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(57) Abstract

Novel oligomeric monoclonal antibodies with high avidity for antigen are described. The oligomers, typically of the IgG class, are secreted having two or more immunoglobulin monomers associated together to form tetravalent or hexavalent IgG molecules. The oligomers can be formed by substantially duplicating regions of the light chain, particularly the variable region. Oligomeric antibodies of the IgG isotype cross the placenta and can provide passive immunity to a fetus, which is particularly important for protecting newborns against pathogens such as group B streptococci.

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OLIGOMERIC IMMUNOGLOBULINS

FIELD OF THE INVENTION

The present invention relates to the field of immunodiagnosis and immunotherapy, and more particularly, to novel oligomeric forms of immunoglobulins.

BACKGROUND OF THE INVENTION

Since the advent of monoclonal antibody technology there has been a veritable explosion in diagnostic and therapeutic utilities for antibodies. The diagnostic uses now range from highly sensitive and specific *in vitro* immunoassays to *in vivo* imaging. Therapeutically, monoclonal antibodies are being formulated to treat a wide variety of cancers and infectious diseases, to regulate a patient's immune response, and to even serve as immunogens, as with anti-idiotypic antibodies.

As antibody technology and genetic engineering procedures have advanced, researchers have focused on ways to modify antibody structure and, in some cases, antibody function. For instance, it has now become possible to engineer a different binding affinity for an antibody, to switch an antibody class, to change the species of an antibody, or to add heterologous non-immunoglobulin polypeptides to an antibody chain.

Structurally, antibodies are composed of one or more units, or monomers, each typically portrayed as resembling a Y shape. A monomer contains four polypeptides - two identical copies of a polypeptide known as the heavy (H) chain, and two identical copies of a polypeptide called the light (L) chain. The two heavy chain polypeptides are approximately 440 amino acids long and about 55,000 daltons each. The two light chains are

approximately 220 amino acids long and each about 25,000 daltons. One light chain associates with one heavy chain, and any one antibody molecule will have only one type of light chain and one type of heavy chain. The amino-terminal variable region of a light chain associates with the amino-terminal variable region of one heavy chain to form an antigen binding site. The carboxy-terminal regions of the two heavy chains fold together to make the F_C domain. The four polypeptide chains of the resulting immunoglobulin molecule are held together by disulfide bridges and noncovalent bonds.

Antibodies are divided into classes - IgG, IgM, IgA, IgE and IgD - on the basis of the type of heavy chain polypeptide they contain (γ, μ, α, ϵ, and δ, respectively). There are only two types of light chain proteins, kappa (κ) and lambda (λ). The classes also vary in the number of monomers that join to form complete antibody. For example, IgM antibodies have five monomers, each with two antigen binding sites, yielding ten identical antigen binding sites for each molecule. IgM is thus referred to as decavalent. IgG, IgE, and IgD typically consist of a single monomeric unit and thus are bivalent, and IgA may consist of one, two or more monomers.

An antibody's intrinsic affinity is a measure of the strength of its binding to an epitope. In principle, it represents the binding by one antigen binding region, i.e., one-half of a monomer's total binding sites. Avidity, on the other hand, is a measure of the overall stability of the complex between antibody and antigen. Avidity is effected by the intrinsic affinity of the antibody for the epitope, the valency of the antibody and antigen, and the geometric arrangement of the interacting components.

Oligovalent interactions may allow low affinity antibodies to bind antigen tightly and can greatly stabilize immune complexes. Thus, antibodies of high

avidity may possess increased therapeutic or diagnostic value compared to similar antibodies of low affinity and low avidity. Unfortunately, it is not always possible to produce monoclonal antibodies with the desired avidity. For instance, although it may be possible to produce monoclonal antibodies to a particular epitope, they may not be IgMs, or the IgMs may be of low affinity. Further, IgMs may possess traits which are undesirable for an intended application. For instance, compared to IgGs, IgMs do not cross the placenta and may possess shorter stability, decreased shelf life, shorter in vivo half-life, and may also be difficult to produce in bulk.

Pollock et al., Proc. Natl. Acad. Sci. USA 85:2298 (1988), have described antibodies with increased binding ability from among a population of antibodies with low binding ability. The authors showed that the structural changes in the antibody responsible for the altered activity occurred in the heavy chain constant region, and that the increased binding activity was not due to polymerization of the antibody.

Accordingly, what is needed in the art are antibodies with high avidity yet which avoid many of the difficulties inherent in working with IgMs. Such antibodies would yield more successful, stable interactions with the target antigen and thus may be more useful in therapeutic or diagnostic settings. Quite remarkably, the present invention fulfills these and other related needs.

SUMMARY OF THE INVENTION

The present invention is concerned with the discovery of novel oligomeric monoclonal antibodies produced by a cell and which comprise two or more immunoglobulin monomers. In one aspect of the invention the novel antibodies possess increased avidity for antigen when compared to that of a bivalent antibody from

which they may be derived. In another aspect the oligomeric antibodies have a γ - heavy chain and thus are of the IgG isotype, and may comprise from two to about six or more monomeric units. In certain embodiments the light chains are kappa. The light chains may have an insertion of amino acid sequences, such as a duplication of a variable or constant region domain and thus may be of a molecular weight substantially greater than the parental molecule from which it may be derived. Thus at least one of the monomers which are associated by the cell into multimers may have one aberrant and one normal light chain or two aberrant light chains.

In one particular embodiment the invention comprises a oligomeric monoclonal antibody capable of binding antigen, which oligomeric antibody is produced by a cell and comprises two or more immunoglobulin monomers of the same antigen binding specificity and each having gamma heavy chains. The multimers are associated noncovalently and the heavy chain is of the gamma-1 subclass and the light chain is of the kappa class. The genes which encode the variable regions of the heavy and light chains may be obtained from a donor cell line different from a cell line that serves as a source of the light and heavy chain constant region genes. In this embodiment the heavy and light chains are primarily human. The oligomeric immunoglobulin of one embodiment binds group B streptococci and is substantially more protective <u>in vivo</u> than a parental IgG monomeric antibody from which it is derived.

In other embodiments the invention concerns methods for producing the oligomeric monoclonal antibodies. Where the multimer is of an IgG isotype, for example, the method comprises transfecting into immortalized host cells the linked genes which code for a light chain, which may be either kappa or lambda. The host cell also expresses a heavy chain, which may be endogenous to the cell line or linked genes coding for

the heavy chain constant and variable regions may be transfected into the cell, as for the light chain. The light and heavy chain variable region genes are typically derived from the same donor cell line so as to be capable of forming a functional antibody to the selected antigen. The gene which encodes the heavy chain constant region, however, may be from the same or different donor cell line. The transfected host cells are then cultivated and the culture supernatants screened for the presence, in the case of gamma heavy chain transfectants, of an antibody with a relatively higher avidity than a normal monomeric antibody from which it may be derived and/or with a molecular weight greater than about 150,000 kD. The supernatant may also be initially screened for a light chain with an increased molecular weight, but subsequent screenings for, e.g., increased avidity, would also be necessary. Transfected cells which secrete the oligomeric antibody are identified and cloned, if necessary, and the antibody is subsequently recovered from culture supernatants.

In another embodiment the invention comprises a method of generating oligomeric antibodies which are particularly useful in treating disease. For example, a oligomeric IgG which binds to the B carbohydrate of group B streptococci (Streptococcus agalactiae) may be used to prevent or treat infection by this organism. In one application the antibody may be used to prevent fetal or neonatal infections, where the antibody composition is administered to the pregnant female prior to or during the birthing process and the oligomeric antibody passes through the placenta and into the bloodstream of the fetus.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1a and 1b show the structures of pNγ1A2.1 and pNκA1.1, respectively. In Fig. 1a, the γ1 constant region gene is shown as an insert into the BamH I site of pN.1, with the variable region gene shown as an insert into the Hind III site of the constant region gene. Arrows above the immunoglobulin genes show the direction of transcription. In Fig. 1b, the kappa immunoglobulin gene is shown as an insert in the BamH I site of pN.1, where the arrow above the immunoglobulin genes show the direction of transcription.

Fig. 2A is an analytical chromatogram from gel filtration chromatography of unfractionated 1B1 monoclonal antibody; 2B and 2C are chromatograms of the high and low molecular weight fractions, respectively;

Fig. 3 is a non-SDS PAGE size analysis in buffers containing urea of size fractionated 1B1 monoclonal antibody; lane 1 is IgG1 monomeric human monoclonal antibody, lane 2 is the low molecular weight fraction of 1B1, lane 3 is the high molecular weight fraction of 1B1, and lane 4 is unfractionated 1B1;

Fig. 4A is a PAGE analysis of unfractionated 1B1 monoclonal antibody reduced with ß-mercaptoethanol, where lane 1 is antibody 1B1, lane 2 is normal IgG1 transfectoma-derived anti-GBS human monoclonal antibody, and lane 3 is normal IgG2 transfectoma-derived anti-GBS human monoclonal antibody; Fig. 4B is a PAGE analysis with the samples of Fig. 4A, except without reducing agent; Fig. 4C is a two-dimensional gel using antibody 1B1 un-reduced in the first dimension and reduced in the second dimension;

Fig. 5 shows the size fractionation of protein A- purified 1B1 monoclonal antibody under reducing conditions, with fractions then electrophoresed on SDS-polyacrylamide gels and protein bands detected with a silver stain;

Fig. 6 is the structure of $pG_{\gamma}2$-A2H, where the $_{\gamma}2$ constant region gene is shown as an insert into the BamH I site of pG, and the variable region gene, A2H, is shown as an insert into the Hind III site of the constant region gene; the arrow above the immunoglobulin gene shows the direction of transcription, and the EcoR I site is indicated for restriction prior to transfection;

Fig. 7 shows the results of binding assays using semi-purified group B strep antigen which demonstrate the avidity of the IgG2 oligomer, 6F5, compared to normal monomeric IgG2 (8B8), IgG1 monomer (D3), 1B1 IgG1 oligomer, and parental 4B9 IgM;

Fig. 8 shows the strategy for sequencing the aberrant light chain V region, using the primers indicated;

Fig. 9 depicts a cloning strategy followed for constructing a duplication of the L'V exon but not the promoter and leader, where E is EcoR I, X is Xba I, S is Sac I, H is Hind III, Bg is Bgl II, C is Cla I, N is Not I, Sp is Sph I, and P is Pvu I;

Fig. 10 shows the construction of pGk.5 which has the C_k region for the 4B9 monoclonal antibody, into which the duplicated V_k genes were cloned to form pGkA1.12;

Fig. 11 shows a non-SDS PAGE size analysis of vector pGkA1.12 derived antibody 23B1 treated with urea, where lane 1 is antibody 23B1 unfractionated, lane 2 is antibody 1B1 low molecular weight fraction (99% monomer), lane 3 is antibody 1B1 high molecular weight fraction (85% dimer), and lane 4 is unfractionated 1B1 antibody (15% dimer);

Fig. 12 shows the insertion of a nine amino acid linker at the N-terminus of the duplicated variable region;

Fig. 13 shows the results of binding experiments using monomeric and oligomeric IgG 1B1 and an IgM (16/2B) with similar binding specificity and using

group B streptococci as antigen. Binding was assayed with an anti-human heavy chain specific antibody;

Figs. 14A and 14B show binding of an anti-idiotypic antibody to the IgM parental antibody 4B9 (Fig. 14A) and to the 1B1 multimer compared to the 1B1 IgG monomer (Fig. 14B). In Fig. 14A, the open square represents binding of 4B9 suspended in PBS/Tween/human sera diluted 1:40; the closed diamond represents 4B9 in specimen diluent (2.5% w/v nonfat dry milk, 0.01% thimerosal, 0.005% anti-foam A, in 20mM sodium citrate) and sera diluted 1:40; the open circle represents 4B9 in PBS and Tween; the open diamond represents 4B9 in specimen diluent; and the closed square represents a human IgM of a binding specificity different from 4B9, diluted in specimen diluent, as a control for the specificity of the anti-idiotype antibody. In Fig. 14B, the open square represents oligomeric 1B1; the closed diamond represents oligomeric 1B1 in a 1:100 dilution of human serum; the closed square represents the oligomeric 1B1 in a 1:500 dilution of human serum; and the open diamond represents the monomeric form of 1B1;

Fig. 15 is an in vitro functional analysis of monomeric and oligomeric IgG in an opsonophagocytic assay, where 1B1 antibody monomer is open circles, 1B1 antibody oligomer is closed circles, and open triangles are the IgM t4B9;

Fig. 16 is the DNA and deduced amino acid sequences of the 1B1 light chain, where L'V(1) and L'V(2) refer to the first and second copies of the L'V regions;

Fig. 17 is the sequence obtained from sequencing the original 4B9 V region clone; and

Fig. 18 is the sequence of the heavy chain variable region for the 4B9 human monoclonal antibody.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The ability of an antibody to protect against challenge with a pathogen often depends on the ability of an antibody to bind antigen with sufficient avidity that it can initiate the complement cascade. An antibody of the IgG class, for example, because of the divalent binding property of IgG molecules, may not have sufficient avidity for an antigen to be protective, whereas an IgM of the same affinity and antigenic specificity, because of the decavalent nature of IgM molecules, may be protective. However, the use of certain classes of antibodies for therapeutic or diagnostic purposes may be limited by the intrinsic properties of those antibody classes. For example, IgM antibodies normally lack the ability to pass through the placenta, whereas IgG antibodies can pass transplacentally and thereby confer protection to the fetus. This feature is an especially important consideration in developing monoclonal antibody-based products for treating diseases, particularly infections, associated with fetuses and newborns. The present invention provides an approach to solving these problems through the generation of novel oligomeric immunoglobulins.

The oligomeric immunoglobulins may have any of the heavy chains and subclasses thereof associated with the species of the antibody being utilized, so long as the heavy chains in any one monomer which contributes to form the oligomer are of the same class. Particularly preferred are gamma heavy chains, so as to form oligomeric IgG molecules, but alpha, mu, epsilon or delta type heavy chains or subclasses known for each species of animal may also be employed. For instance, the subclasses of human IgG include types 1, 2, 3 and 4, whereas subclasses or murine IgG include types 1, 2a, 2b and 3. By oligomeric or multimeric is meant an antibody having more monomer units (i.e., H2L2) than known for

said isotype, which units are associated covalently or noncovalently so as to yield functional antigen binding sites. Typically, IgGs, IgDs and IgEs are reported to comprise single monomers, while IgMs have five monomers and IgAs usually have one, but may have two or more monomeric units associated covalently via a J chain. The light chains may be either kappa or lambda. In an embodiment described below, a preferred multimer has heavy chains of the gamma class (human) and a kappa light chain.

The oligomeric immunoglobulins may be of any species or combination thereof from which monoclonal antibodies may be prepared. While murine and human immunoglobulins are most commonly utilized, other species such as lagomorpha, bovine, ovine, equine, porcine, avian or the like may be employed. For therapeutic administration to humans, substantially human immunoglobulins are preferred to minimize their recognition as foreign by a patient's immune system. It should be understood that the monoclonal antibody art and genetic engineering techniques have advanced sufficiently such that antibody sequences of one species may be interchanged with those of another species. Thus, as used herein, a "human" antibody, for example, refers to one that is primarily human in origin but may also contain some non-human and/or non-immunoglobulin sequences. Similarly, when referring to "immunoglobulin," it will be understood that some non-immunoglobulin sequences may be present in the molecule while retaining the ability to bind antigen.

The oligomeric immunoglobulins will be biosynthetically produced, i.e., by a cell line or cell extract, as distinguished from the chemical conjugation of antibodies using well known laboratory procedures, such as by employing cross-linking reagents. The multimers may be conveniently produced starting with an established cell line which secretes monoclonal

antibodies that bind to a desired antigen or epitope thereon. The parental antibody-producing cell line may be isolated from B cells of several species using conventional fusion, viral transformation or other immortalization techniques well known to those skilled in the art. For instance, human monoclonal antibodies may be generated using Epstein-Barr virus (EBV) transformation, hybridoma fusion techniques, or combinations thereof. See, for example, Kozbor et al., Proc. Natl. Acad. Sci. USA 79:6651 (1982), and U.S. Pat. Nos. 4,464,465 and 4,624,921, which are incorporated herein by reference. The parental monoclonal antibody may be of any of the classes or subclasses of immunoglobulins. By monoclonal antibody is meant an antibody produced by a clonal, continuous cell line separate from cells which produce antibodies of a different antigen binding specificity. Thus such monoclonal antibodies are produced and isolated from other monoclonal antibodies and, accordingly, in substantially pure form (relative to other antibodies) and at a concentration greater than normally occurring in sera from the animal species which serves as the B cell source. Alternatively, using PCR or similar techniques V regions can be cloned from transformed or non-transformed cells.

The genes which encode the variable regions of the light and heavy chains may be cloned from the parental cells and transfected into a host cell capable of antibody expression. Genes which encode an aberrant light chain as described herein could also be transfected into cells which express immunoglobulin of the appropriate specificity, or cell which express only the corresponding H chain. The host cell is a eucaryotic cell, preferably mammalian, which is capable of providing post-translational modifications to immunoglobulin proteins, including leader sequence removal, correct folding and assembly, glycosylation at correct sites, and

secretion of functional antibody from the cell. Lymphocyte lines are preferred hosts, especially those of a B cell lineage. The host cell may be a myeloma line, such as Ag8.653 as described below.

Transfection of host cells may be accomplished by a number of means, such as electroporation, calcium phosphate coprecipitation of DNA, DEAE dextran precipitation, protoplast fusion and microinjection. Following transfection the cells are typically incubated for a brief period in nonselective medium and are then transferred to selective medium and observed for proliferation. After a sufficient time for cell outgrowth, the supernatants are screened for oligomeric immunoglobulin, as described below.

To facilitate identification and selection of transfectants that express the transfected immunoglobulin genes, a gene that confers a selectable phenotype (a selectable marker) is generally introduced into the cells along with the immunoglobulin gene(s) of interest. Preferred selectable markers include genes that confer resistance to drugs, such as neomycin, kanamycin, methotrexate and mycophenolic acid. The selectable marker may be an amplifiable selectable marker. Selectable markers are reviewed in Thilly, Mammalian Cell Technology, Butterworth Pub., Stoneham, MA, and the choice of such a marker is well within the level of ordinary skill in the art. Selectable markers may be introduced into the cell on the same plasmid as the gene(s) of interest or on a separate plasmid. See generally, U.S. 4,634,665, incorporated herein by reference. If on the same plasmid the selectable marker and the gene of interest may be under the control of different promoters or the same promoter.

The gene which encodes the light chain variable region may be cloned with or without the genes which encode the remainder of the light chain, i.e., the constant region. If only the variable region is cloned,

it is inserted into a cassette containing the C region of the appropriate light chain class, such that the genes are operably linked so as to encode a complete light chain when transfected into a host cell. The C region gene may be from the same or different parental cell line as the V gene. The cassette may contain transcriptional enhancer elements, promoters, etc. to direct higher levels of expression. The cassette may also contain a selectable marker for convenience in identifying and selecting successful transfectants.

The structure of human kappa light chain genes is described in Hieter et al., Cell 22:197-207 (1980), Hieter et al., J. Biol. Chem. 257:1516-1522 (1982), and in Klobeck et al., Nucleic Acids Res. 12:6995-7006 (1984), which are incorporated herein by reference. In the germline configuration there is a single kappa constant region gene per haploid genome. Upstream of the constant region is a group of 5 J kappa region genes, and the variable region genes are presumably located further upstream. To clone a rearranged kappa variable region gene, a probe may be used which encompasses the germline J kappa gene. With certain restriction enzymes which do not have recognition sequences between the J region and constant region (e.g. BamH I), a C kappa probe can also be used to detect rearranged kappa variable region genes.

The structure of human lambda light chain genes is described in Hieter et al., Nature 294:536-540 (1981), and in Udey and Blomberg, Immunogenet. 25:63-70 (1987), which are incorporated herein by reference. To clone the rearranged lambda genes, a C-lambda probe is used to detect rearrangements occurring 5' of it at the J-lambda locus, taking advantage of the close proximity of J-lambda and C-lambda genes.

In kappa chain producing cells, one or both kappa alleles may be rearranged, but the lambda locus is rarely or never rearranged. In lambda chain producing cells, one or both lambda alleles may be rearranged and

both kappa alleles are usually rearranged, although in a non-productive manner such that a complete kappa chain cannot be expressed. As with the heavy chains, one cannot determine at the DNA level (and may not be able to determine at the RNA level) which rearranged light chain is expressed at the protein level. Therefore, either both rearranged kappa or both rearranged lambda genes should be cloned from kappa or lambda producing cells, respectively, and the functional gene determined by transfection experiments.

To clone a human light chain gene, genomic DNA obtained from a donor cell of interest is restricted with an appropriate endonuclease and DNA fragments of the desired size (determined by Southern transfer using an appropriate probe such as J-kappa, C-kappa, or C-lambda) are enriched by preparative sucrose gradient centrifugation and used to make a bacteriophage library. The library is then screened with the appropriate probe and positive plaques identified, purified and amplified. Bacteriophage DNA is then isolated from the plaques.

If the rearranged light chain variable region and constant region genes are found on a single restriction fragment small enough to be cloned into a single vector they may be cloned together and need not be subcloned into an expression cassette before being transfected into host cells. In other instances, the variable and constant region genes may not fit into one vector and the variable region gene will require subcloning into an expression cassette containing the constant region gene. In still other instances it may be possible to clone the variable and constant region genes on a single vector but it may be desirable to transfer the genes into a cassette containing other components which may enhance the level of expression.

To facilitate restriction mapping and subcloning into an expression vector or cassette, the rearranged light chain variable region genes (VJ

elements) may first be subcloned. Plasmid DNA containing the rearranged genes may be identified by hybridization with the appropriate J or C region probe. The variable region gene insert is then transferred into the desired expression vector by restriction of vector and gene insert with endonucleases producing compatible ends and then ligation of the two components. Bacteria may be transformed with the ligation mixture and transformants containing the complete light chain genes (variable and constant region) in proper orientation may be identified using restriction digestion. Once the genes coding for the light chain have been assembled in an expression vector (either bacteriophage or plasmid), the vector may be linearized and transfected, either simultaneously or sequentially, with genes coding for the corresponding heavy chain into a suitable eucaryotic host cell capable of expressing the genes.

It should be understood that the resultant light chain which is assembled into the antibody monomers may be considered "aberrant," in that it contains additional amino acid sequences, such as, for example, a duplication of light chain sequences, particularly that of the variable region. The inserted amino acids should be of a sufficient length and in an appropriate position in the variable region of the light chain to allow the aberrant light chain to effect oligomerization. Typically, the insert will be greater than about 20 contiguous amino acids, although a larger insert might yield a more effective polymerization of the monomers to form oligomers. An insert of more than about 50 amino acids is preferred, including a substantial duplication (70% or more) of a V region, e.g., about 75 up to 110 amino acids or more. Thus, the insert is desirably a derivative of a sequence typically associated with an antibody, and particularly antibody light chains, but other sequences which induce polymerization can also be employed. Most preferably, the sequence is derived from

the light chain V region, and may or may not include V region signal sequences.

The additional sequences are desirably inserted in a region of the light chain (kappa or lambda) in a position that does not substantially interfere with the ability of the light chain to associate with the heavy chain or to bind antigen. Thus, any insert should conserve the framework regions of the light chain, i.e., the contact residues between the heavy and light chains variable regions. Most preferably, the insert of additional sequences which result in oligomerization will be between the V region and the C region or amino to the V region of the light chain.

Linker amino acids can also be used to facilitate oligomerization. Linkers may be sequences of from about three to up to ten or more amino acids chosen so as to provide structural flexibility to the inserted sequences. For instance, in one preferred embodiment described herein the linker is a nine amino acid sequence comprised of four residues from the proteolytic enzyme Factor Xa and a sequence of five relatively neutral amino acids, primarily Gly and Ser. Typically the linkers will be inserted at the N-terminus of the inserted (e.g., duplicated V region) sequence or at the C-terminus.

Although the precise mechanism by which oligomerization occurs is not completely understood, and without intending to be limited to a particular mechanism, the extra light chain sequences from one chain may associate with a similar aberrant light chain on another IgG monomer. In an alternative mechanism, the extra sequences from one variable region may displace the corresponding variable region from a normal or aberrant light chain of another IgG monomer and thereby associate with the variable region of the H chain of that monomer. Thus, while the molecular weight of a light chain is usually about 25,000 daltons, an aberrant light chain may be up to 10% larger, sometimes 20%, and more usually up

to 50% larger or more (thus up to 37,000 daltons or more). Such aberrant, light chain-like molecules will be recognized by typical anti-light chain reagents, such as anti-kappa immunoglobulins. One or two of such light chains may be incorporated into at least one monomer which is used by the cell (or extract) to form oligomers. The oligomers (or multimers) may include at least one monomer having such an aberrant light chain.

The mechanism by which both the normal and abnormal light chains described herein may be synthesized by the same cell is not completely understood. One hypothesis, offered by way of possible explanation but not limitation, follows. The two polypeptide chains can be made from either multiple copies of the vector integrated into the host cell genome or by alternative splicing of an RNA transcript from a single copy of the vector. If there are multiple copies of the vector, one or more of these copies may have been altered before, during or after the integration process. These alterations include, but are not limited to, the duplication of all or part of one or both of the exons encoding the light chain, particularly that of the variable region. In the case of alternative splicing mechanisms, the vector sequences may be integrated in such a way that cryptic splice acceptor sites within the vector may become activated and be joined with splice donor sequences from either the vector or from the host genome. Both the normal and abnormal splicing may occur in the same cell at differing efficiencies.

The variable region from a heavy chain may be cloned and inserted into a cassette containing constant region genes from the same or a different cell line. The constant region may be of the same or different isotype or subclass as the constant region of the parental antibody, and may be of the same or different species. Operably linked genes may then be used to transfect the

host cell line which expresses the compatible light chain.

The general structure of heavy chain genes is well known. See, e.g., Ellison and Hood, Adv. Human Genet. 13:113-147 (1983), Joho et al., Curr. Topics Develop. Biol. 18:15-58 (1983), and Calame, Ann. Rev. Immunol. 3:159-195 (1985), which are incorporated herein by reference. Genes coding for a functional heavy chain consist of a rearranged variable region gene separated by an intron from one of any of the constant region genes, gamma, mu, alpha, epsilon or delta or subclasses thereof.

It may be desirable to construct cassettes consisting of heavy chain constant region genes in DNA vectors such that a variable region gene can be inserted 5' of the constant region gene to produce an expression vector containing a complete, expressible heavy chain gene. This may be accomplished by inserting the heavy chain constant region gene into a vector with a short polylinker 5' of the constant region gene. The variable region gene may then be inserted in the polylinker region. Polylinkers which provide sites for restriction endonucleases are well known in the art.

A preferred vector for the construction of an expression cassette, as used in the examples below, is pSV2-neo, described in Southern and Berg, J. Mol. Appl. Genet. 1:327-341 (1982), incorporated herein by reference. pSV2-neo has a pBR322 origin of replication, a gene encoding beta-lactamase, an SV40 origin of replication to allow it to replicate in mammalian cells, and a neomycin-kanamycin resistance gene which provides for a selectable marker in bacterial and mammalian cells. Other vectors suitable for stably transferring genes into a variety of cells are well known and are described in, for example, Coffin, RNA Tumor Viruses, vol. 2, Weiss et al., Eds., Cold Spring Harbor Laboratory, New York (1985), pp. 36-63, and Kwok et al., Proc. Natl. Acad.

Sci. USA 83:4552 (1986), which are incorporated herein by reference.

In constructing a cassette for expression of immunoglobulin genes it may be necessary to modify the vector so that it does not contain restriction sites which might interfere with later cloning steps. Modifications may also be designed to direct higher levels of expression of the gene encoding the selectable marker, thereby facilitating the selection of cells taking up the vector.

In some instances, depending on the vector into which the heavy chain constant region (CH) gene is inserted, it may be convenient to first subclone the CH gene into a shuttle vector in an orientation such that the 5' end is adjacent to a polylinker region. The shuttle vector may then be digested with an appropriate restriction enzyme to release the CH gene with the polylinker at the 5' end. The isolated fragment may then be cloned into an expression vector to form a convenient cassette for inserting the desired V region gene in the polylinker region.

Clones of CH genes of various human isotypes have been described. Cloning of the germline human gamma-1 gene is reported in Flanagan and Roberts, Nature 300:709-713 (1982), and Ellison et al., Nucl. Acids Res. 10:4071-4079 (1982). The gamma-2 gene is also described in Flanagan, id., and in Ellison and Hood, Proc. Natl. Acad. Sci. USA 79:1984-1989 (1982), and Takahashi et al., Cell 29:671-679 (1982). The gamma-3 and gamma-4 genes are described in Flanagan, id., and the gamma-4 gene is also described in Ellison, id., and Ellison et al., DNA 1:11-18 (1981). The mu gene is described in Ravetch et al., Cell 27:583-591 (1981), and the alpha-1, alpha-2 and epsilon genes are described in Flanagan, id. Each of the foregoing articles are incorporated herein by reference.

Either one or both heavy chain alleles can undergo VDJ rearrangement, but only one of the RNA

species (if both are expressed as mRNA) is translated into a complete heavy chain polypeptide (allelic exclusion). Thus it may be necessary to clone both rearranged alleles and then determine which one is expressed by transfection into appropriate host cells. To accomplish this, genomic DNA is restricted with an appropriate endonuclease, DNA fragments of the desired size (determined by Southern transfer and hybridization with a JH region probe) are enriched by preparative sucrose gradient centrifugation and used to make a bacteriophage library, which is then screened with a JH region probe and positive plaques identified, purified and amplified. To facilitate restriction mapping and subcloning into a CH cassette the rearranged heavy chain variable region genes (VDJ genes) may be subcloned. Plasmid DNA containing the rearranged V region genes is identified by hybridization with a JH probe and by a conserved 0.8 kb Hind III-Bgl II fragment 3' of the J regions. The V region gene is then transferred into the CH cassette at a position 5' of the CH gene by restriction of each component with endonucleases producing compatible ends and then ligating the two components. Bacteria may be transformed with the ligation mixture and transformants containing the complete heavy chain gene in proper orientation may be identified using restriction digestion. Once the entire heavy chain gene has been assembled it may be linearized and then transfected into a suitable host for co-expression with a light chain gene.

A wide variety of recombinant DNA techniques are available to the skilled artisan to isolate and transfer the DNA encoding the oligomeric antibodies or regions thereof, including regions contributing to the oligomeric association of monomers, to a variety of hosts for production. Techniques for producing chimeric, class-switched or humanized (hypervariable region transfers) antibodies, as well as antibodies with

modified constant regions, in addition to procedures described herein, are described in copending U.S. Serial No. 254,004, in U.S. Pat. 4,816,397, and in EPO publications EP 173,494 and EP 239,400, which are incorporated herein by reference.

For instance, such techniques may be employed to produce $F(ab')_2$ fragments of oligomers. $F(ab')_2$ fragments which comprise aberrant light chains can be produced using recombinant techniques, where the aberrant light chain is produced as described above, and the heavy chain is terminated prematurely by inserting stop codons following the cysteine residues responsible for inter-chain disulfide bonding. In the case of, e.g., the human $\gamma 1$ chain this can be accomplished by introducing a translation stop codon in reading frame following the cysteine at residue 226 (numbering according to Eu, Edelman et al., Proc. Natl. Acad. Sci. USA 63:78-85 (1969) by, for example, using site-directed mutagenesis. This approach has the advantage of minimally altering the RNA structure so normal RNA splicing and stability can be preserved. The first cysteine residues involved with inter-heavy chain disulfide bonding for human IgGs occur at residues 221, 226 and 226 for IgG_2, IgG_3, and IgG_4, respectively.

Detection of immunoglobulin multimers can be by a variety of techniques, including liquid chromatography, gradient centrifugation, and gel electrophoresis, among others. Increased activity of the multimer can be measured by quantitative antigen binding assays, antibody competition experiments and opsonophagocytic assays. These techniques are familiar to those skilled in the art, and are described in, for example, Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor, N.Y., (1988), incorporated by reference herein. As described above, the host cell may secrete a variety of immunoglobulin molecules. If the host is a myeloma it may or may not inherently produce an antibody of a

binding specificity different from that of the transfected genes. The cells may secrete bivalent monomers in addition to multivalent multimers. The multimers may have at least one monomer wherein one or both light chains of the monomer are aberrant, as described above. Thus the antibody in a culture supernatant may contain as much as 50% or more normal monomer, and as little as 5-10% multimer. Desirably, the multimer will be at least 10% of total antibody, preferably at least 25%, more preferably at least about 50%, and most preferably 90% or more of total antibody produced by the cell culture.

Antibodies with increased binding avidity will be useful in the treatment and diagnosis of a wide variety of conditions. If a oligomeric antibody is derived from a parental monoclonal antibody-producing cell line, the multimer may offer improved therapeutic and diagnostic characteristics. For instance, if the parental antibody is an IgM molecule and the multimer is composed of IgG monomers, the multimer may possess therapeutic anti-infective qualities that are inherent to certain multivalent antibodies such as IgMs, and also have qualities inherent to IgG monomers, such as their unique ability to cross the placenta and, for example, protect a fetus from infection. The IgG multimers may also possess attributes typically associated with IgGs, such as ease of purification, increased stability, increased shelf life, and increased half-life in vivo.

Due to the increased avidity of the multimers, it is now possible to convert a previously non-protective antibody to be protective against infection or tumors, for example, or to act as an immunomodulator by potentiating or otherwise regulating a host's immune response to a particular antigen. Where a parental antibody is a non-protective IgG, a multimer produced using the methods herein may provide sufficient avidity to confer a significant protective ability. Of course,

it is understood that the present invention is not limited to antibody multimers which are protective or show other such functional attributes in vivo, as increased avidity also makes feasible an array of diagnostic procedures perhaps not otherwise available to a bivalent parental molecule of low affinity and/or low avidity.

It is also understood that the invention is not limited by the antigen binding specificity of the particular multimers exemplified herein. A wide variety of monoclonal antibodies have been described in the technical and patent literature, many of which are publicly available from cell line depositories. The methods described herein provide the ability to produce novel oligomeric compositions from immunoglobulin genes from such cell lines.

The ability of the resultant antibodies to inhibit a tumor, to act as an immunomodulator, or to protect against challenge by a pathogen, for example, can be measured in a wide variety of in vitro and in vivo systems, as will be known to the artisan. An exemplary protocol to test for enhanced protection against group B streptococci, using a oligomeric antibody derived from an IgG which was non-protective or weakly protective, when administered after birth, appears in Example VIII below.

The novel oligomeric monoclonal antibodies and pharmaceutical compositions thereof are particularly useful for parenteral administration for prophylactic and/or therapeutic treatment. Preferably, the pharmaceutical compositions can be administered parenterally, i.e., subcutaneously, intramuscularly, or intravenously. Thus, this invention provides compositions for parenteral administration which comprise a solution of the oligomeric monoclonal antibody or a cocktail of oligomeric and non-oligomeric antibodies dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used,

e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc. The concentration of antibody in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intravenous infusion to treat an infection in an adult could be made up to contain 250 ml of sterile Ringer's solution, and about 100 mg to 10 grams of antibody. Actual methods for preparing parenterally administrable compounds will be known or apparent to those skilled in the art and are described in more detail in for example, Remington's Pharmaceutical Science, 16th ed., Mack Publishing Company, Easton, PA (1982), which is incorporated herein by reference.

The compositions containing the present oligomeric antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend on the severity of the infection and the general state of the patient's own immune system, but generally range from about 0.1 to about 50 mg of antibody

per kilogram of body weight per dose, with dosages of from 5 to 25 mg of antibody per kilogram per patient being more commonly used. It must be kept in mind that the materials of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by substantially human oligomeric antibodies made feasible by this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or cocktails thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per kilogram, especially 0.5 to 2.5 mg per kilogram. A preferred prophylactic use is for treatment of fetuses and neonates at risk from infection through their mothers. When treatment is dependent on passage through the placenta, the dosage may require adjustment to reflect the percentage of antibody which is able to pass from the blood of the pregnant female to that of the fetus.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of oligomeric antibody of this invention sufficient to treat the patient.

The antibodies of the present invention can find further use in vitro in diagnostic assays. By way

of example, the oligomeric IgG antibody of Example I below can be used for detecting the presence of group B streptococci, for vaccine preparation, or the like.

For diagnostic purposes, the antibodies may be either labeled or unlabeled. Unlabeled oligomeric antibodies may find particular use in agglutination assays, or they may be used in combination with other labeled antibodies (second antibodies) that are reactive with the oligomeric antibody, such as antibodies specific for the Fc regions. Alternatively, the antibody may be directly labeled. A wide variety of labels may be employed, such as radionuclides, particles (e.g. gold, ferritin, magnetic particles, red blood cells), fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are known to those skilled in the art, such as competitive and sandwich assays as described in, e.g., U.S. Pat. 4,376,110, incorporated by reference herein, and Harlow and Lane, supra.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of the presence of a selected antigen. Thus, the subject antibody compositions of the present invention may be provided, usually in lyophilized form in a container, either alone or in conjunction with additional antibodies. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about .0001% wt., based on the antibody concentration. Frequently it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the

excipient may be present in from about 1 to 99% of the total composition. Where a second antibody capable of binding to the oligomeric antibody is employed in an assay, this will be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following examples are offered by way of illustration, not by limitation.

EXAMPLE I

Production of a High Molecular Weight Antibody with Increased Binding Avidity

Monoclonal antibodies specific for the group B carbohydrate of group B streptococci (GBS) have been previously isolated and characterized, as described in copending U.S. Patent Application No. 189,359, incorporated herein by reference. These antibodies, originally of the IgM isotype, were class switched to IgG using recombinant DNA techniques, as described in copending application USSN 254,004, incorporated herein by reference.

A monoclonal antibody having a molecular weight substantially greater than a typical IgG antibody was produced using V region genes cloned from the parental 4B9 lymphoblastoid cell line (LCL) using techniques similar to those employed in class-switching procedures referred to above. Briefly, mouse myeloma line Ag8.653 was first transfected with a plasmid vector encoding the light chain (kappa) of 4B9. Clones expressing the light chain were then transfected with a vector encoding the heavy chain. Transfectoma clones secreting immunoglobulins of increased antigen binding ability were found to produce IgG molecules and light chains of increased molecular weight.

A. General Procedures

1. Preparation of Genomic DNA

Total high molecular weight DNA was purified from 1-2 x 10^8 cells of 4B9 in a method similar to the procedure of Perucho et al., 1981, Cell. 27:467-76. Briefly, the cells were washed by centrifugation in PBS (phosphate buffered saline) or TBS (Tris buffered saline) and then lysed in a Tris buffer containing SDS, EDTA and proteinase K. Following proteinase digestion, the DNA was extracted with phenol and chloroform, ethanol precipitated, and resuspended in TE (10 mM Tris, pH 7.8, 1 mM EDTA). The sample was then digested with RNAse A, and again extracted with phenol and chloroform, ethanol precipitated and resuspended in TE. The procedure is described below in more detail.

Approximately 1-2 x 10^8 4B9 cells were obtained from cultures and centrifuged at 1,000 x g for 10 minutes. The cells were resuspended in 50 ml TBS (8 g NaCl, 0.38 g KCl, 3.0 g Tris base per liter, and 1 N HCl to pH 7.4), or PBS, centrifuged, resuspended, and centrifuged again. After the final centrifugation, the cells were resuspended in a small amount of PBS or TBS. In some cases, they were stored at -20°C. For each 10^8 cells, 5 ml of lysis buffer (10 mM Tris, pH 8.0, 50 mM EDTA, 150 mM NaCl) containing 200 µg/ml Proteinase K (Boehringer Manheim) was added. This volume is henceforth considered to be one volume. A one-fortieth volume of 20% SDS was added, the tube quickly mixed and then incubated overnight at 37°C. One volume of 2X phenol extraction buffer (10 mM Tris, pH 8.0, 12 mM EDTA, 650 mM NaCl) was then added, followed by two volumes of phenol (equilibrated with Tris to neutral pH) containing 0.1% 8-OH quinoline. The tubes were covered, mixed gently for 5 to 10 minutes, then centrifuged at 2500 rpm for 20 minutes. The aqueous phase was transferred to a

fresh tube and the phenol extraction repeated 1 to 3 times until the interface had disappeared. The aqueous phase was again transferred to a fresh tube and two volumes of phenol:chloroform:isoamyl-alcohol in a ratio of 25:24:1 (PCI) added. The tube was mixed, centrifuged, and the aqueous phase transferred to a fresh tube. The PCI extraction was repeated 1 to 3 times until the interface had disappeared. The aqueous phase was again transferred to a fresh tube and extracted twice with chloroform, isoamyl alcohol (24:1). Finally, the aqueous phase was transferred to a fresh tube and gently overlaid with four volumes of ethanol. The tube was gently mixed by inversion until the DNA had formed a tight clot. The DNA was removed with a glass rod and rinsed sequentially with 70% ethanol in ethanol dilution buffer (10 mM Tris, pH 8.0, 10 mM EDTA, 150 mM NaCl), 85% ethanol and 100% ethanol. The DNA was dried by absorption of the ethanol on a paper towel and resuspended in a few ml TE buffer overnight at 37°C. This volume is henceforth considered to be one volume.

One volume of 2X RNAse A buffer (100 mM Tris, pH 8.0, 20 mM EDTA, 20 mM NaCl) was added, followed by RNAse A (Sigma, #R-5125) to 100 μg/ml (the RNAse A had previously been boiled for 30 minutes to remove trace DNAse activity). This mixture was incubated for 3 hours at 37°C. One-fifth volume of 3 M sodium acetate was added, the DNA extracted twice with phenol/chloroform, precipitated with ethanol and resuspended in TE as described above.

As a source of germline DNA, high molecular weight DNA was prepared from cells of human placenta. Connective tissue was removed from about 5 grams of the placental tissue; the tissue was minced with a scalpel and then rinsed three times by centrifugation and resuspension in TBS, each at 1,000 x g at 4°C for 10 minutes each. The tissue (3.5 g) was resuspended in 20 ml TBS, homogenized in a motor driven glass-teflon

homogenizer, centrifuged again for 10 minutes at 1,000 x g, and resuspended in 3.5 ml TE. This formed a very thick suspension. Lysis buffer was added (0.5 M EDTA, 0.5% Sarkosyl, and 100 μg/ml Proteinase K) and incubated at 50°C for 1 hour, then overnight at 37°C. The remainder of the procedure was as described by Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, page 280, which is incorporated by reference herein, except that following RNAse A digestion and prior to extraction with phenol, the sample was again digested with Proteinase K (100 μg/ml) overnight at 37°C. The final concentration of placental DNA was 0.15 mg/ml.

2. Preparation of Bacteriophage λL47.1

In these experiments, the bacteriophage λL47.1 was used as a vector. E. coli strains LE 392 (ATCC number 33572) and 803 supF (obtained from Dr. N. Hozumi, Mt. Sinai Hospital, Toronto, Ontario) were used as hosts for bacteriophage growth. λL47.1 is described in Maniatis et al., Molecular Cloning, A Laboratory Manual, p. 41 and Loenen and Brammer, 1980, Gene, 10:249. To use λL47.1 as a vector the middle "stuffer" fragment was removed, thereby allowing for the incorporation of foreign DNA. Briefly, this was accomplished by ligating the cohesive ends of the vector, digesting the vector with an appropriate restriction endonuclease that removed the stuffer, and then separating the bacteriophage arms from the stuffer piece by centrifugation through a sucrose gradient.

The experimental procedure was as follows. The cohesive ends of 100 μg λL47.1 DNA were annealed by incubation in buffer containing 10 mM $MgCl_2$ and 150 mM Tris, pH 8.0, for 1 hour at 42°C. The annealed ends were then ligated and the ligase then heat inactivated. For the preparation of Hind III arms, the vector DNA was then digested to completion with both Hind III and BamH I. The latter enzyme served to further digest the stuffer

piece such that its ends (BamH I) were no longer compatible with those of the vector arms (Hind III). The vector was then treated with bovine alkaline phosphatase so that the bacteriophage arms could not ligate to each other. The DNA was then phenol and chloroform extracted, ethanol precipitated and resuspended in TE buffer.

Fifty micrograms of the digested bacteriophage DNA were then layered onto a 40 ml linear 10-40% sucrose gradient in 20 mM Tris, pH 8.0, 1.0 M NaCl, 2.5 mM EDTA. The tubes were centrifuged at 26,000 rpm for 20 hours at 20°C in a SW28 rotor (Beckman). The gradient was tapped from the bottom and fractions containing vector arms, but no stuffer piece, were identified by electrophoresing a small sample through an agarose gel. DNA was recovered from these fractions by ethanol precipitation and resuspension in TE buffer.

In some instances an alternate but less efficient procedure was utilized. Briefly, bacteriophage arms were prepared by digesting λL47.1 DNA with Hind III, annealing of the cos sites, separating by sucrose gradient centrifugation, and pooling of fractions which contained phage arms but not the stuffer fragment. The phage arms gave a high background, so the DNA was further digested with BamH I to cut any contaminating stuffer fragment. This significantly reduced the background seen during ligation and packaging without the addition of any insert DNA.

3. Preparation of DNA Hybridization Probes

All DNA hybridization probes described herein were used as inserts isolated from either phage or plasmid vector DNA and were labeled by nick translation using a kit provided by Bethesda Research Labs (Nick Translation Reagent Kit, cat. no. 8160SB) and ^{32}P-dCTP from New England Nuclear or Amersham. Typically, the probes had specific activities of 1-2 x 10^8 CPM/μg. Prior to hybridization, the probe was denatured for 10

minutes at about 95°C, quickly chilled on ice, then added to the hybridization mix. Unless otherwise noted, hybridizations were carried out as follows: filters were prehybridized in a basic mix (50% formamide, 5X SSC, 5X Denhardt's solution, 50 μg/ml sheared salmon sperm DNA, 50 mM Hepes, pH 6.8, 5 mM EDTA, and 10 μg/ml poly (A)) supplemented with 1% glycine and 0.1% SDS for at least 2 hours in sealed plastic bags in a 42°C water bath. The hybridization buffer varied according to the type of hybridization; for genomic Southern Transfers, the basic mix was supplemented with 10% dextran sulfate, 0.2% SDS, and 10^6 CPM/ml probe; for Southern transfer of cloned DNA, the basic mix was supplemented with 0.2% SDS and 10^5 CPM/ml probe; for first screens of phage libraries, the basic mix was supplemented with 0.2% SDS and 10^6 CPM/ml probe, while subsequent screens frequently had less probe. Hybridizations were normally performed overnight at 42°C. Following the hybridization, filters were first washed in 2X SSC, 0.1% SDS at room temperature and then in 0.1X SSC, 0.1% SDS at 65°C. Filters were exposed to X-ray film with intensifying screens at -70°C for varying times. When the signal was obviously very strong the intensifying screens were omitted and the film was exposed at room temperature.

B. Light Chain Gene Constructs

Since both kappa light chain alleles from cell line 4B9 could be expressed at the RNA level, both rearranged kappa light chain alleles were cloned and it was then determined by transfection which one was translated into a protein capable of combining with the appropriate heavy chain to bind antigen.

Southern Transfer analysis with a kappa J region probe (Jκ) and a kappa constant region probe (Cκ) was used to determine the size of BamH I fragments of genomic DNA from cell line 4B9 that would contain rearranged kappa chain VJ genes. It was important to

determine that the BamH I fragments were of sufficient size to contain the complete variable region gene as well as sufficient upstream sequences to promote adequate levels of transcription. This size was determined by measuring the distance (in kb) from the BamH I site 3' of Cκ to the 5'-most Jκ gene, and adding approximately 2 kb for L-V gene and promoter sequences. (In many Vκ genes, there is a BamH I site within the L-Vκ complex; in such cases, alternative enzymes such as Hind III or EcoR I can be used.) The Vκ and Cκ genes were found on the same BamH I fragments of less than 19 kb and could therefore be cloned into a single phage vector, λL47.1. The Vκ and Cκ genes were therefore cloned together on a single restriction fragment.

A bacteriophage library was made from BamH I-digested and size fractionated genomic DNA from LCL 4B9. The library was screened with Jκ and Cκ probes, DNA was prepared from positive plaques, and digestions were performed on phage DNA to confirm the identity of the clones as kappa genes. The phage DNA was then subcloned into an expression cassette.

1. Kappa Gene Cloning

One hundred twenty micrograms of DNA from cell line 4B9 and 10 μg of human placental DNA were digested with BamH I and then 7.5 μg of each fractionated by electrophoresis through a 1.1% agarose gel in TAE buffer (0.04 M Tris acetate, 0.001 M EDTA). Southern transfers were performed on DNA digested with BamH I which was then probed with a Jκ probe which spans the Jκ region. The Jκ probe was obtained from a plasmid containing the 1.8 kb Sac I fragment of a germline human kappa clone described by Hieter et al., 1980, Cell 22:197-207 and J. Biol. Chem. 257:1516-1522 (1982). This probe hybridized to an 11 kb germline fragment in placental DNA and to a 16 kb rearranged fragment of 4B9 DNA. Southern transfer experiments using the same probe, but either EcoR I or

Hind III digests, showed that there were two rearranged kappa genes in 4B9, suggesting that BamH I digestion had produced two fragments of similar molecular weight such that they were not distinguishable from each other.

Fifty micrograms of BamH I-digested 4B9 DNA were fractionated on a 10-40% sucrose gradient, and fractions containing DNA of the desired size (16 kb) were screened by Southern transfer analysis with the Jκ probe. Those hybridizing most strongly were used for the library construction.

Approximately 5% of the DNA in the peak fractions was ligated with λL47.1 phage arms, (the DNA having been digested with BamH I) using T4 DNA ligase and a ligation buffer (50 mM Tris, pH 7.8, 10mM $MgCl_2$, 1mM DTT, 6mM KCl, 1mM ATP and 1mM spermidine) containing 5% PEG. The ligation reaction was then packaged in vitro, as described below for the heavy chain gene cloning. Titers of the bacteriophage were determined using the 803 supF strain of E. coli and were usually between 0.5 and 1.5×10^5 total plaque forming units (pfu) from each ligation reaction (corresponding to 5% of a sucrose gradient fraction). Approximately 10^5 pfu were plated out. Duplicate plaque lifts were taken from each plate using nitrocellulose paper (Schleicher and Schuell, BA85). One set of filters was hybridized with the Jκ probe, and the other was hybridized with a Cκ probe (a 2.7 kb EcoR I fragment derived from the same germline human kappa clone as previously described). Only plaques hybridizing with both probes were picked and further purified as described for the heavy chain variable region gene cloning.

Two kappa clones, designated A1κ and A2κ, both having approximately 16 kb BamH I inserts were identified. Phage DNA from both clones was cotransfected with γ_2 heavy chain cassettes (containing both heavy chain V regions) into Ag8.653 cells. Culture supernatants were assayed for IgG2 expression. Only

cells transfected with A1k and the A2 heavy chain allele secreted IgG of the correct specificity. This showed that the A1k allele was the one found in the 4B9 antibody.

The entire BamH I fragment of bacteriophage DNA Alκ was subcloned into the BamH I site of the pN.1 vector, described below, to form pNκA1.1.

The myeloma line Ag8.653 was then transfected with pNκA1.1 by electroporation, using 10 μg of pNκA1.1 which had been linearized with Apa I and about 2 x 10^7/ml myeloma cells. Cells were incubated in complete media for 48 hours and then transferred to microtiter wells at 10^4 cells/well in RPMI containing 20% FCS and 0.25 mg/ml G418 (Gibco 860-1811IJ). Cells were fed every 4 to 5 days. Two to three weeks later, the cells were assayed for the presence of intracellular human kappa chain by fluorescence staining. Three master wells stained positive, which then were cloned in soft agarose. Soft agarose clone 7G-B4 gave a fluorescence intensity of 4+ and 100% of the cells were positive. The cells were plated in 24 well dishes and antibody was quantitated at 1.7 μg/ml using a whole antibody reference. Clone 7G-B4 was again cloned by soft agarose and secondary clone 7G-B4-1 gave a 4+ fluorescence intensity. This line was referred to as "B4-1".

B. Heavy Chain Constant Region Gene Construct, pNγ1.1

A heavy chain cassette containing a human γ1 heavy chain constant region gene was constructed. A phage clone, Phage 3A (Ellison et al., 1982, Nucleic Acids Research, 10:4071-4079) was used as a source of the germline Cγ1 gene. This clone had been obtained by screening a human fetal liver genomic DNA library with a γ1 cDNA constant region probe, as described above.

Briefly, the γ1 cassette, containing non-coding sequences both 5' and 3' of the coding region of the γ1 constant region gene, was made by first subcloning the

appropriate sequences of the Cγ1 gene into the polylinker region of the pUC vector to produce pγ1.1. The Cγ1 sequence was then removed from the vector along with the desired polylinker sequences and inserted into pN.1 (described below) to produce pNγ1.1. The detailed procedures used to construct the cassettes follow.

Two micrograms of Phage 3A DNA were digested to completion with BamH I, extracted with PCI, ethanol precipitated and resuspended in 20 μl TE buffer (10 mM Tris, pH 7.8, 1mM EDTA) (0.1 mg/ml). 10 μg of pUC18 were digested to completion with BamH I, PCI extracted, ethanol precipitated and resuspended in 44 μl H_2O. The DNA was then treated with calf intestinal alkaline phosphatase (CIP; 28 units, from Boehringer-Mannheim), PCI extracted, ethanol precipitated and resuspended in TE to 50 ng/μl. 200 ng of the BamH I digested phage DNA was then ligated to 200 ng of the BamH I digested and phosphatased pUC18 with 400 units of T4 DNA ligase (New England Biolabs) for 15 minutes at room temperature in ligation buffer in a final volume of 20 μl. The reaction was then diluted to 80 μl with ligation buffer lacking polyethylene glycol (PEG). 160 units of T4 DNA ligase (New England Biolabs) was added and the mixture incubated overnight at 15°C. 5 μl of the ligation mixture was then used to transform 200 μl of competent JM83 cells. The transformed bacteria were plated on LB-agar containing ampicillin (100 μg/ml) and X-gal as a color indicator. White colonies were picked and streaked onto a plate containing LB-agar and ampicillin. These colonies were grown and then screened by colony hybridization, using a nick-translated γ3 probe, an EcoR I-Hind III insert derived from p3.6RH4.2 described by Krawinkel, EMBO J. (1982) 1:403-407 and Flanagan and Rabbits, 1982, Nature, 300:709-713.

DNA was prepared from those colonies which hybridized with the probe. The DNA was digested with BamH I, electrophoresed, photographed and transferred to

nitrocellulose and hybridized with the same γ3 probe. DNA containing a 10.5 kb insert was detected and the resulting vector was designated pγ1.1.

The next step in the construction of the Cγ1 heavy chain cassette involved removing the Cγ1 sequence from the vector and inserting it into the vector pN.1 to form the heavy chain cassette pNγ1.1.

The vector pN.1 was constructed from the pSV2-neo vector, ATCC No. 37149, described by Southern and Berg, J. Mol. Appl. Genet. (1982) 1:327-341. The pSV2-neo vector was modified so that it did not contain a Hind III site which would interfere with later cloning steps. Briefly, this was accomplished by digesting pSV2-neo with Hind III, making the termini blunt ended with the Klenow fragment of DNA polymerase, self-ligating the DNA and using it to transform competent bacteria. The resulting vector was designated pN.1.

To insert the Cγ1 sequence into the pN.1 vector, 2.4 μg of pN.1 was first digested with 99 units of BamH I, phenol extracted, ethanol precipitated and resuspended in 50 μl TE (giving 0.05 μg/μl). pγ1.1 was also digested with BamH I. Approximately 1 μg of the pγ1.1 digest was combined with 100 ng of the pN.1 digest in ligation buffer in a volume of 98 μl and heated to 65°C for 5 minutes. 2 μl of T4 DNA ligase (New England Biolabs) was added and the sample was incubated for 30 minutes at room temperature followed by overnight incubation at 14°C. 10-50 μl of the ligation reaction were used to transform 200 μl of competent DH5α cells. The bacteria were plated on LB-agar containing 100 μg/ml ampicillin and 200 μg/ml kanamycin. Transformants were screened for the presence of plasmid DNA containing a 9-11 kb BamH I insert. Transformants containing an approximately 9.6 kb insert in pN.1 were selected. The DNA was also digested with Hind III and Bgl II to confirm the presence of the γ1 insert and to determine its orientation. Hind III linearized the DNA. Bgl II

digestion produced fragments of 2.1 kb and approximately 10 kb. This is consistent with the γ1 gene being in the opposite orientation to the neo gene if a deletion exists which includes the Bgl II site 5' of the γ1 coding region. Hind III plus Bgl II digestion produced bands of 2.1 and a doublet at about 6 kb. This is consistent with the orientation and deletion. The vector isolated from these transformants was designated pNγ1.1.

1. Insertion of Heavy Chain Variable Region Gene into Heavy Chain Constant Region Cassette

Both rearranged heavy chain alleles from the cell line 4B9 were cloned and inserted into a cassette containing the γ_2 heavy chain constant region in the appropriate orientation. It was determined by transfection that the A2H allele was translated into a polypeptide chain that combined with the appropriate light chain to ultimately bind antigen (see copending USSN 254,004, incorporated herein by reference). The details are as follows.

Southern Transfer analysis with a human J_H region probe, pJ_H was used to determine the size of Hind III fragments of DNA from cell line 4B9 that would contain the rearranged heavy chain VDJ genes. It was important to determine that the Hind III fragments were of sufficient size to contain the complete variable region gene as well as sufficient upstream sequences to promote adequate levels of transcription. This was determined by measuring the distance from the Hind III site in the J_H-Cμ intron to the 5' most J_H element, and adding approximately 2kb for L-V and promoter sequences.

A bacteriophage library was made from Hind III digested and size-fractionated genomic DNA from cell line 4B9. The library was screened with the J region probe, DNA was prepared from the positive plaques and the variable region gene was then subcloned into pUC18 to facilitate mapping of restriction sites and identification of duplicate clones. The variable region

genes of 4B9 were then inserted into the pNγ1.1 vector to form an expressible heavy chain gene.

2. Preparation of 4B9 Genomic Heavy Chain Variable Region DNA Insert

One hundred-twenty micrograms of DNA from LCL 4B9 prepared as described above and 10 μg of human placental DNA were separately digested with Hind III and 7.5 μg of each were fractionated by electrophoresis through a 1.1% agarose gel in TAE buffer. The DNA was transferred to nitrocellulose paper using standard procedures, baked overnight at 80°C in a non-vacuum oven and hybridized with a ^{32}P-labelled human heavy chain J region probe (pJ_H insert). The probe insert (in pBR322) extends from an artificial EcoR I site near the 5' end of the J region to the Hind III site in the major intron between the J region and the mu constant region. The DNA sequence of the J region and part of the intron from a similar clone is described in Ravetch et al., 1981, Cell, 27:583-591 and in Rabbitts et al., 1983, Nature, 306:806-809. Since immunoglobulin genes must undergo a rearrangement to be expressed, DNA fragments were sought which were present in the 4B9 DNA but absent in the germline (placental) DNA. Either one or two rearranged bands were expected, depending on whether one or both alleles had undergone rearrangement, respectively. The pJ_H probe hybridized to a 9 kb germline fragment in placental DNA, and to 10.6 and 7.8 kb rearranged fragments of 4B9 DNA.

Fifty micrograms of the Hind III-digested 4B9 DNA was fractionated on a 10-40% sucrose gradient as described above for preparation of vector DNA arms. Fractions containing DNA of the desired size were screened by Southern transfer analysis with the ^{32}P-labeled pJ_H insert probe. Since the 10.6 and 7.8 kb fragments were easily separable, those fractions most

enriched for each band were used for the library construction.

Approximately 5-10% of the DNA in the peak fractions was ligated with the λL47.1 phage arms, prepared as described above, using T4 DNA ligase and ligation buffer. The ligation reaction was then packaged in vitro. Packaging extracts were prepared according to the procedure of Grosveld et al., 1981, Gene, 13:227-237, using a freeze-thaw lysate prepared from E. coli strain BHB 2688 (ATCC No. 35131) and a sonic extract prepared from strain BHB 2690 (ATCC No. 35132). Other procedures or commercial packaging extracts may be used. Titers of the bacteriophage were determined using the 803 supF strain of E. coli and were usually between 0.5 and 1.5×10^5 total plaque forming units (pfu) from each ligation reaction (corresponding to 5-10% of a sucrose gradient fraction).

Each library corresponding to a particular band seen on Southern transfer analysis consisted of at least 10^5 plaques (derived from one to three sucrose gradient fractions). The libraries were screened using ^{32}P-labeled pJ_H insert. Positive plaques were picked, diluted, plated, and screened using the same probe. This process was repeated (usually a total of 3 to 5 times) until all plaques on a plate were positive.

3. Subcloning the Heavy Chain Variable Region Gene into pNγ1 Cassette

The 4B9 heavy chain variable region gene was inserted into the pNγ1 cassette described above. Specifically, the pNγ1.1 vector was digested with Hind III and then treated with CIP. Hind III-digested phage A2H (as described above) was used as the source of the 4B9 variable region gene. Vector DNA was then mixed and ligated with phage DNA and the ligation mixture was used to transform competent DH5α cells. Transformants were screened for the presence of the variable region gene and

the orientation of the gene was determined by various restriction enzyme digestions. Thus, a construct containing the 4B9 variable region gene adjacent to a γ1 constant region gene was prepared and designated pNγ1A2.1. The procedures for the production of the pNγ1A2.1 construct follow.

7.5 μg of A2H phage DNA was digested with 80 units of Hind III, ethanol precipitated and resuspended in 150 μl TE. 50 μg of pNγ1.1 was digested with 200 units of Hind III, treated with CIP, ethanol precipitated and resuspended in 100 μl TE (0.5 μg/μl). 500 ng of the A2H DNA was then ligated with 25 ng of pNγ1.1 DNA using 1 unit T4 DNA ligase (Boehringer-Mannheim) in a reaction volume of 20 μl for 30 minutes at 37°C. 5 μl of the ligation mixture was diluted with 30 μl TE and used to transform 60 μl competent DH5α cells. The transformed cells were plated on LB-agar containing ampicillin at 100 μg/ml.

Twelve colonies were picked and DNA was prepared from them. Digestion of the DNA with Hind III showed the presence of an insert of the appropriate size (about 8.4 kb). Orientation was then determined by digestion with BamH I and Bgl II. Based on the absence of the Bgl II site 5' of the γ1 gene, transformants which produced bands of 2.0, 3.7, 6.1, 4.8 and 7.8 kb were selected. The resulting construct was termed pNγ1A2.1.

4. Transfection with Heavy Chain Construct pNγ1A2.1

The heavy chain construct was then transfected into the B4-1 cells which expressed the kappa light chain. Since the light chain vector (pNk1A1.1) contained a gene conferring G418 resistance to the cells, it was necessary to cotransfect the heavy chain vector with another vector containing a different selectable marker, the Ecogpt gene. pG.3, a derivative of pSV2-gpt and described in Serial No. 254,004, was used for this purpose. Approximately 5 x 10^7 B4-1 cells were

electroporated in the presence of 10 μg pNγ1A2.1 (restricted with BamH I) and 1 μg of pG.3. 48 hours post electroporation, cells were plated in microtiter plates at 2 x 10^4/well (2 x 10^5/ml) in RPMI 1640/10% FCS containing 1 μg/ml mycophenolic acid, 15 μg/ml hypoxanthine, and 250 μg/ml xanthine. Two to three weeks later, the culture supernatants were assayed for IgG production, and cells from 10 of the wells were submitted to soft agarose cloning. Cell line 1B1-D3-F3-D2-F3 was subjected to two rounds of soft agarose cloning. The resulting clone was termed "1B1". Prior to filing this patent application the 1B1 transfectoma was deposited with the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD, 20852 and assigned ATCC accession number CRL 10293, deposited Nov. 7, 1989.

The 1B1 transfectoma produced IgG1 monoclonal antibody which appeared to have higher binding avidity (using microtiter wells coated with GBS) than other cell lines resulting from the same transfection. FPLC analysis of the antibody product demonstrated that the majority of the monoclonal antibody produced had a typical molecular weight of 150 kD, while 6-15% of the antibody produced had a molecular weight of 300-450 kD. Thus, the 1B1 transfectoma produced IgG_1 monoclonal antibody in two molecular weight forms.

Protein A purified 1B1 monoclonal antibody was used for further analysis by gel filtration chromatography. Nutrient exhausted supernatant from 1B1 cells was passed over a protein A affinity column (Langone, J. Immunol. Meth. 55:277 (1982)). Antibody was eluted with 0.1 M citrate buffer, pH 3.5 (0.02% sodium azide) and was dialyzed against PBS, pH 7.2. Fig. 2A is an analytical chromatogram of unfractionated 1B1 monoclonal antibody on a Superose® 6 FPLC column (1 x 25 cm) linked in series with a Superose® 12 column (1 x 25 cm). Twenty micrograms of antibody in 100 μl was loaded and run at 0.30 ml/min in PBS buffer. The monoclonal

antibody resolved as two peaks: low molecular weight material eluted as expected for monomeric IgG (160-180 kD), and high molecular weight fractions eluted in the range expected for IgG multimers (>300 kD). IgG monoclonal antibodies from non-related transfectomas resolved as single monomeric peaks.

For preparation of monomeric and oligomeric IgG, the protein A purified antibody (146 mg) was chromatographed in PBS on two Superose® 12 columns (1.6 cm x 50 cm) and one Superose ® 6 column (5 cm x 50 cm) linked in series. Starting with 146 mg of protein A purified monoclonal antibody, 5.2 mg of high molecular weight and 70 mg of low molecular weight fractions were pooled, concentrated and adjusted for A_{280}, and analyzed by FPLC as for the first sample. Fig. 2B and Fig. 2C are chromatograms of the high and low molecular weight fractions, respectively. The high molecular weight pool contained about 85% high molecular weight material and 15% low molecular weight material, while the low molecular weight pool was about 99% homogeneous. The monoclonal antibodies from the two pools were tested for antigen binding and biological activity, as discussed further below, and the high molecular weight material showed greatly enhanced activity.

EXAMPLE II
Immunochemical Analysis of High Molecular Weight Monoclonal Antibodies

Immunochemical approaches were used to determine the physical association of IgG molecules in the high molecular weight fraction. Antibody from each pool was electrophoresed on polyacrylamide gels under standard Laemmli non-reducing polyacrylamide gel electrophoresis (PAGE) conditions. The antibodies from

the pools did not resolve into high and low molecular weight bands corresponding to the size exclusion data.

Non-SDS PAGE employing 0.5 M urea was performed in a low pH discontinuous buffer system as follows. A stacking gel consisting of 2.5% acrylamide, 0.1 M KOH, 0.5 M urea, pH 6.8 was poured onto a 3 to 5% linear gradient resolving gel buffer containing 60 mM KOH, 0.37 M acetic acid 0.5 M urea, pH 4.3. For both the stacking and resolving gels the total acrylamide monomer to bis ratio was 19:1. The reservoir buffer contained 0.35 M β-alanine, 0.14 M acetic acid, pH4.5. The gels were polymerized onto Gelbond PAG film for support. Samples were incubated at room temp. in stacking buffer containing 10% glycerol for 1 hr. prior to electrophoresis.

Under these conditions, as shown in Fig. 3, the high molecular weight antibody appeared predominantly as dimer (twice the molecular weight of monomer) and minor monomer and trimer components, while the low molecular weight pool was essentially all monomer. Thus, it was concluded that the IgG dimers were held together by non-covalent interactions sensitive to dissociation under some (≥ 2% SDS) but not all (0.5 M urea) denaturing conditions.

Purified unfractionated 1B1 monoclonal antibody was analyzed under reducing and non-reducing conditions on polyacrylamide gels in the presence of SDS and revealed two unusual banding patterns. The electrophoresis of protein A purified monoclonal antibody samples reduced with 1% β-mercaptoethanol was performed on a 5-15% linear gradient SDS-polyacrylamide gel as described by Laemmli, followed by protein detection using Coomassie Blue R-250. With reducing agent and SDS, silver stained gels (Damerval, et al., Electrophoresis 8:158 (1987)) showed the presence of typical L and H chains (25 and 50 kD bands, respectively) in both 1B1 and control monoclonal antibodies. However, 1B1 contained an

additional 37 kD band (Fig. 4A). Immunoblots showed the 37 kD and 25 kD bands reacted with kappa L chain specific reagents, but not with H chain specific reagents. The 37 kD band was designated the "aberrant" L chain, or "L_a." The original L chain producing cell line (B4-1 cells) made both L chains and other transfectants derived from this cell line also contained two L chains and produced varying amounts of dimer.

When the unfractionated 1B1 monoclonal antibody was electrophoresed without reducing agent (Fig. 4B), it resolved into three bands of distinct molecular species. The lowest species corresponded to a molecular weight of 180 kD, and had about the same mobility as normal IgG_1. The middle species corresponded to a molecular weight of 190 kD. The highest molecular weight species corresponded to an immunoglobulin of molecular weight approximately 203 kD.

The antibody in each band was further electrophoresed through a reducing, 2-dimensional SDS polyacrylamide gel. The first dimension was the unreduced sample of monoclonal antibody 1B1 as described above; after electrophoresis, the sample lane was excised, boiled in 1% ß-mercaptoethanol for 10 min., and washed twice for 5 min. each in running buffer. The gel strip was placed into the stacking gel area above a 5-15% linear gradient SDS-polyacrylamide gel (1.5 mm), and was held in place by stacking gel. The gel was run and silver stained as above.

The results of running the three molecular weight antibodies in the unreduced/reduced 2-dimensional PAGE are shown in Fig. 4C. All three species demonstrated a similar protein band corresponding to the typical heavy chain molecular weight. However, the three species contained different amounts of the two light chain bands, one of normal molecular weight (25 kD), and the other of a greater molecular weight (37 kD). Both

heavy and light chain bands were verified as such by reaction with anti-light and anti-heavy chain antibodies.

These data suggested that at least three species of monomeric and oligomeric antibodies exist in the population produced by the 1B1 cell line, where all three can have relatively normal H_2L_2 IgG stoichiometry: two normal heavy chains associated with two normal light chains ($H_2L_nL_n$) for the 180 kD band; two normal heavy chains associated with one normal and one large aberrant light chain ($H_2L_nL_a$) for the 190 kD band; and two normal heavy chains associated with two large aberrant light chains ($H_2L_aL_a$) for the 203 kD band.

The role of L_a in forming the oligomeric antibody molecules was further examined. Protein A purified 1B1 antibody was separated on an FPLC size exclusion column, and protein containing fractions collected between 29 and 40 minutes were analyzed by reducing PAGE. Silver-stained gels showed that virtually all samples possessed three chains with molecular weights of 25, 37 and 50 kD (Fig. 5). However, the high molecular weight material (fractions 29-34) had an increased proportion of L_a (37 kD) to L_n (25 kD). These data suggest that L_a was associated with oligomer formation, although all immunoglobulin molecules possessing L_a did not necessarily form oligomers.

EXAMPLE III

Preparation of IgG2 Oliogmers

To further confirm the contribution of the L_a chain to oligomer formation, two additional types of transfectomas were prepared. A cell line producing IgG_1 was made using the same H chain vector and a recipient cell line which produced only normal L chain. A cell line producing IgG_2 was made by transfecting a $\gamma 2$ vector

with the same H chain V region gene (i.e., 4B9) into the B4-1 cell line which expressed both the L_a and L_n. The IgG_1 antibody showed no evidence of oligomerization, while the IgG_2 line expressed monoclonal antibody containing 10-15% dimer, similar to the 1B1 cell line, confirming that the L_a was required for high molecular weight oligomer formation.

The IgG_2 transfectoma secreting oligomer was produced as follows. A Hind III fragment from Phage 5A, containing the γ2 gene, was subcloned into pUC18 in the orientation such that the 5' end was adjacent to the polylinker region. This construct was then digested with BamH I, thus releasing the γ2 gene with a polylinker at the 5' end. The isolated fragment was then cloned into the BamH I site of pG forming the cassette pGγ2. pG is a derivative of pSV2-gpt in which the 121 bp Hind III-Bgl II fragment has been removed by digestion, Klenow fill-in and religation.

The heavy chain variable region gene was purified from a Hind III digest by electrophoresis through low melt agarose. The pGγ2 cassette was also digested with Hind III, treated with CIP, and then separated by electrophoresis through low melt agarose. The melted agarose slices containing the linearized cassette and variable region gene insert were combined, the DNA ligated, and then used to transform competent bacteria. Transformants were screened for those containing the V gene inserted in the same orientation as the γ2 gene in the pGγ2 vector.

To identify transformants containing the desired insert, the resulting colonies were inoculated into 2 ml of LB broth containing 1% glucose and 100 μg/ml ampicillin and grown overnight at 37°C. DNA was prepared from the overnight growths and digested with Hind III to determine which samples contained the variable region insert in the pGγ2 vector. Orientation of the insert within the vector was determined by digestion with Bgl II

and BamH I. Samples containing the variable and constant region genes in the same orientation were selected and designated pGγ2-A2H (Fig. 15), and the DNA used for transfection into the B4-1 cell line which expressed both L_a and L_n.

The resulting IgG_2 antibody, designated 6F5, was purified from culture supernatant with protein A. FPLC analysis of purified antibody (FPLC buffer was 10 mM sodium phosphate, .9% NaCl, pH 7.2) showed a high molecular weight shoulder, approximately 12% of total antibody, analogous to the 1B1 antibody produced by transfection of the same cell line secreting aberrant light chain. When analyzed by SDS-PAGE, performed as generally described above except that the gel was 4-12% rather than 5-15%, three species were apparent, similar to the 1B1 antibody: a normal IgG_2 ($H_2L_nL_n$), an IgG_2 molecule with one aberrant light chain ($H_2L_nL_a$), and an IgG2 molecule with two aberrant light chains ($H_2L_aL_a$).

The increased avidity of the higher molecular weight IgG_2 oligomers was confirmed in an antigen binding assay. The assay was performed as generally described in Example VII below, except that GBS antigen rather than whole bacteria was adsorbed to the microtiter wells. Controls included a normal monomeric IgG_2 antibody (8B8) a normal monomeric IgG_1 antibody (D3), and the parental IgM transfectoma antibody (t4B9). The results, shown in Fig. 7, indicate that the IgG_2 oligomeric antibody 6F5 reacted remarkably similar to that of the IgG_1 oligomeric antibody 1B1. Although t4B9 IgM binding was slightly higher, a different second step reagent was employed to detect IgM which may account for some of the increased O.D.

EXAMPLE IV

Sequence Analysis of Aberrant Light Chain

The DNA and amino acid sequences of the aberrant light chain were determined.

L_a and L_n were recovered from SDS-PAGE by electroblotting onto Immobilon™ membrane (Millipore Corp., Bedford, MA) using Mini-transblot Electrophoretic Transfer Cell (Bio-Rad Laboratories, Richmond, CA) as described by Matsudaira, J. Biol. Chem. 261:10035-10038 (1987). The membrane was stained with Coomassie Brilliant Blue, destained and the stained bands (molecular weights 37 kD and 25 kD) were excised with a razor blade for subsequent amino-terminal sequence analysis. Automated Edman degradation was performed in a pulsed-liquid protein sequencer (Model 475A, Applied Biosystems, Inc., Foster City, CA). The phenylthiohydantoin amino acid derivatives were analyzed by reversed phase high performance liquid chromatography using a Model 120A on-line HPLC unit (Applied Biosystems, Inc.) with a PTH C-18 column (2.1 x 220 mm, ABI) and a sodium acetate/tetrahydrafuran/acetonitrile gradient for elution. The results showed that the 21 N-terminal amino acids of both light chains were identical:

Glu-Ile-Val-Leu-Thr-Gln-Ser-Pro-Ala-Thr-Leu-Ser-Leu-(Arg)-Pro-Gly-Glu-Arg-Ala-Thr-Leu

The (Arg) at position 14 was in low yield and also showed some serine. The DNA sequence corresponds to serine.

A V region probe, specific for the 4B9 V_L gene, was then used to screen a cDNA library derived from the 1B1 cell line. The probe was constructed by digesting pUCA1k (5 kb EcoR I - Sac I insert in pUC; see Fig. 1b for the location of the EcoR I(E) - Sac I(S) fragment spanning the VJ gene) with Sac I and Xba I, and the 3 kb band was gel purified on low melt agarose. This DNA fragment includes the sequence "FinalA1k". mRNA was purified from 1B1 cells using the Fast Track™ mRNA

Isolation Kit (Invitrogen Corp., San Diego, CA). A cDNA library was prepared in the pCDM8 vector using oligo(dT) primer and the Librarian™ I Kit (Invitrogen Corp.). The library was screened by colony hybridization using the 4B9 V region probe. Positive clones were sequenced by the dideoxy chain termination method (Sanger) using the strategy shown in Fig. 8. Some of the DNA was sequenced in only one direction because primers from the L'V region annealled at two positions and therefore produced a double ladder. The primers used were as follows:

1 CDM8 reverse	CGACCTGCAGGCGCAGAA
2 4B9 VJ/C sense	TCAACACCGTGACAATTG
3 4B9 840 antisense	CAGATGGTGCAGCCACAG
4 4B9 1080 antisense	TCTCGTAGTCTGCTTTGC
5 T7 forward	AATACGACTCACTATAG

The sequencing revealed that the cDNA clone 4B9-Vk15 contained a repetition of the L'V region exon, resulting in the structure "Leader-L'V-L'V-C," where L'V represents the exon encoding the last three amino acids of the leader sequence and the rearranged VJ gene. The DNA and deduced amino acid sequences of the light chain are as shown in Fig. 16. Note that 4B9- Vk15 is not a complete cDNA clone: it starts with the G of the ATG which encodes the initiator methionine. L'V(1) and L'V(2) refer to the first and second copies of the L'V regions.

The sequence in the L'V regions corresponded to that obtained from sequencing the original V region clone, as shown in Fig. 17. In separate experiments, the heavy chain variable region for the 4B9 human monoclonal antibody was determined, as shown in Fig. 18.

To rule out the possibility of artifacts in the cDNA cloning, the L_a chain amino acid composition was determined. Samples were hydrolyzed for 24 hrs at 110°C with 6N HCl/1% phenol using a Waters PicoTag® Workstation. Analysis was done by precolumn

derivatization with o-phthalaldehyde and reverse phase chromatography on a HP 1090 Liquid Chromatograph. The analysis, shown in Table I, was found to correspond to that predicted by the cDNA sequence.

Table I. Amino acid analysis of aberrant light chains.

	Experimental	Expected	
Amino Acid		Aberrant	Normal
Aspartate/Asparagine	20.7*	22.0	16.0
Glutamate/Glutamine	36.1	35.0	24.0
Serine	40.1	42.0	29.0
Histidine	4.9	5.0	3.0
Glycine	29.7	27.0	15.0
Threonine	27.5	28.0	17.0
Alanine	27.9	27.0	17.0
Arginine	14.8	15.0	9.0
Tyrosine	13.0	14.0	9.0
Cysteine**	ND	6.0	4.0
Valine	19.6	18.0	14.0
Methionine	0.0	0.0	0.0
Isoleucine	11.4	11.0	6.0
Phenylalanine	11.8	12.0	8.0
Leucine	26.1	24.0	16.0
Lysine	14.8	14.0	11.0
Proline**	ND	23.0	14.0

*residues/mole. **not determined.

mRNA of this sequence could be encoded by genomic DNA containing a duplication of either just the L'V exon, or both the leader and L'V exons. In the latter, the second copy of the leader exon would not be included in the mRNA because it lacks a splice acceptor site. Genomic Southern blots of DNA from the 1B1 cell

line were performed and the hybridization intensities of V, C and neo probes in the two copies compared. The V region probe hybridized more intensely to one of the copies, in agreement with the interpretation regarding the duplication of the L'V region.

EXAMPLE V

Construction and Expression of Duplicated L'V Exon to Effect Oligomerization

To further extend the foregoing results, a vector was constructed which encoded a duplication of the L'V exon but not the promoter and leader. Cotransfections were performed using this vector (pGkA1.12) and one for the H chain (pNγ1A2.2) to show the effect on oligomerization when L_a was the only light chain expressed.

The cloning strategy is generally set forth in Fig. 9. Briefly the 3.2 kb Xba1 fragment of pUCA1k.1 was cloned into pIC20R (Marsh et al., Gene 32:481-485 (1984) and the construct referred to as pICA1k.2. The V_k gene in pICA1k.2 was amplified by PCR using the primers A1kPRIM1 (5' primer, sense; CAGCAGAGATCTACAGTTGGTTTGA-TCCTGACTACAT) and A1kPRIM2 (3' primer, antisense; GCCCGAGGATCCGATGAACTAATTTTCTATCCCTTAC). The primers were selected such that the upstream leader and promoter were not a part of the second copy of the V_k gene (the absence of the 5' leader and promoter sequences for V_{k2} will prevent the transcription of 2 different length mRNA, one coding for a single V_k and the other coding for a mRNA with 2 copies of the V_k gene). However, the primers were selected at a position 5' of the V_k gene to maintain the splice site 5' of the V_k gene to allow for the splicing of the second copy of the V_k gene to the first. Furthermore, the primers incorporated a 5' Bgl2 site and a 3' BamH1 site, chosen for ease of cloning into a BamH1

site because of compatible overhangs. Also, this same procedure can also be used to replicate other V_k genes. Moreover, in the correct orientation (of V_{k2}, with respect to the first copy of the V_k gene), the BamH1 site is preserved 3' of the 2 copies of the V_k gene, while in the wrong orientation, the BamH1 site lies between the 2 copies of the V_k gene. When V_{k2} is inserted in the correct orientation, the insert containing the 2 copies of the V_k gene can be excised with a BamH1 + Bgl2 digest, which can in turn be cloned into pGk.5/BamH1 due to compatible overhangs.

The PCR product (V_{k2}) was digested with BamH1 and Bgl2, and cloned into the BamH1 site 3' of the first copy of the V_k gene in pICA1k.2. In the proper orientation, this construct is pICA1k.4. It may be desirable, however, to first clone the PCR product into a vector such as pUC19, then into the construct with the single copy of the V_k gene. This would permit rapid elucidation of the sequence of the PCR product, important due to the nucleotide misincorporation frequency of the Taq1 polymerase used in PCR. In this procedure, the V_{k2} was excised from pICA1k.4 as a Sst1 + BamH1 fragment and cloned into pUC19 to get sequencing information, with the new construct called pUCA1k.2.

As shown in Fig. 9, the 3.8 kb BamH1 + Bgl2 fragment containing the two copies of the V_k gene were cloned into pGk.5/BamH1. This construct also has the C_k for the 4B9 antibody. pGk.5 was constructed as follows, and as shown in Fig. 10: The SV40 enhancer was deleted from pG.3, which is a pSV2-gpt derived vector described in EPO Publication EP 314,161, incorporated by reference herein. pG.3 was digested with Sph I and the sticky ends were filled in with Klenow polymerase. The vector was then digested with Pvu II and ligated. Competent cells were transformed with the ligation product. Plasmid DNA was screened for the absence of Pvu II and Sph I restriction sites. The product was called pG.9.

In the next step, a Not I recognition sequence was inserted into the vector. pG.9 was digested with Nde I, CIP treated and purified on low melt agarose. It was then annealed with a linker containing a Not I recognition sequence and Nde I sticky ends. Following ligation, bacteria were transformed and plasmid DNA was screened for the presence of a Not I site. The DNA was then digested with Not I and recircularlized. This served to remove potential concatamers of the linker. This vector was called pG.10, and has a single copy of the linker. The linkers used are as follows:

5' TAGCGGCCGCA 3'

3' CGCCGGCGTAT 5'

Separately, the pG.12 was constructed. This vector differs from pG.5 by the addition of a Not I site adjacent to the Nde I site. This was accomplished in the same way that pG.9 was prepared from pG.3. pG.5 differs from pG.3 in that it has a polylinker derived from pIC20R replacing the 751 bp found between the EcoR I and BamH I sites.

pG.11 was constructed by combining the polylinker containing Not I-BamH I fragment from pG.12 with the large BamH I-Not I fragment of pG.10, which contains the ampicillin resistance gene, Ecogpt gene, and lacks the SV40 enhancer. This was accomplished by digestion of both vectors with Not I and BamH I and purification on low melt agarose. Following ligation and transformation, DNA was prepared from the transformed bacteria and screened for the presence of EcoR I, Bgl II and Sph I sites from the polylinker, but the lack of a Pvu II and Nsi I site which were deleted from the SV40 enhancer region.

To construct the pGk.4, as shown in Fig. 10, pG.11 was restricted with EcoR I and CIP treated. pCk consists of a 2.7 kb EcoR I fragment containing the human C gene inserted into the EcoR I site of pEMBL18. pCk was digested with EcoR I and the 2.7 kb fragment was gel

purified. The two fragments were ligated and the product used to transform competent JM83 cells. The transformants were screened by colony hybridization with a C_k probe. DNA from positive colonies was screened for the presence of 6.5, 0.5 and 0.125 kb fragments upon digestion with Sac I.

As shown in Fig. 10, pGk.4 was restricted with Cla I and Hind III and then treated with CIP and the large fragment was purified on low melt agarose. pICMHEXX, which consists of a 1 kb Xba I fragment containing the mouse heavy chain enhancer inserted into the Bgl II site of pIC19R (both Xba I and Bgl II sites were filled in with Klenow polymerase prior to ligation of the two fragments) was restricted with Cla I and Hind III and the 1 kb fragment containing the enhancer was purified on low melt agarose. The two fragments were ligated and the product was used to transform competent JM83 cells. DNA from the transformants was screened for the presence of 4.3, 2.7, 0.7 and 0.3 kb fragments upon digestion with EcoR I and Hind III and pGk.5 identified.

The vector resulting from the cloning of the 3.8 kb BamH I + Bgl II fragment from pICA1k.4 (Fig. 9) containing the two copies of the V_k gene into pGk.5/BamH1 was designated pGkA1.12, which was used to cotransfect Ag8.653 cells with a vector which encoded the H chain, pNγ1A2.2.

Culture supernatants from the transfectants were screened for binding to group B polysaccharide. Those binding most strongly were analyzed in the urea-PAGE system for the presence of oligomers. Antibody in nutrient exhausted supernatant from one master well, designated 23B1, was purified as in Example II above and was analyzed in the urea-PAGE system as described in Example II. Following electrophoresis, the gel bond was removed (Albaugh et al., Electrophoresis 8:140 (1987)), and the protein in the gel was transferred to nitrocellulose paper using the reservoir gel buffer

containing 20% methanol (starting at 400 mA, for 18 hrs.) and was immunoblotted as described in Raff et al., J. Infect. Dis. 157:118 (1988). The results, shown in Fig. 11, indicated that dimer and higher oligomers can be synthesized by cells expressing L_a and H only, and no L_n.

EXAMPLE VI

Insertion of a Linker Between L'(2) and VJ(2) Gene

The duplicated variable region was synthesized with a linker at its N-terminus to facilitate the flexibility of the amino terminal V region to potentially facilitate or increase formation of oligomers.

In this example nucleotide sequences which encoded a nine amino acid insert, which comprised a four amino acid recognition sequence for the proteolytic enzyme Factor Xa (Ile-Glu-Gly-Arg) and a five amino acid linker sequence (Gly-Ser-Gly-Gly-Ser), was inserted between the L'(2) and the VJ(2) gene of the kappa light chain coding for the antibody to GBS described above, as generally shown in Fig. 12. A two-step PCR protocol was used to facilitate the insertion of the DNA sequence. The initial template used was pICA1k.2 digested with Hind III. In the first step, two separate reactions were used. In the first reaction, a 5' sense primer (A1kPRIM1, supra), and the 3' antisense primer (A1kPRIM6; AGACTGTGTCAACACAATTTCGCTACCACCGCT) were used. A1kPRIM1 has nonhomologous 5' sequences coding for a Bgl II site 5' of the V gene, and A1kPRIM6 has nonhomologous 5' sequences coding for the 27 nucleotide insert. For the second reaction, A1kPRIM2 (supra) was the 3' antisense primer with 5' nonhomologous sequences coding for a BamH I site, and A1kPRIM7 (ATCGAGGGTAGAGGTAGCGGTGGTAGCGAAATTGT GTTGACACAGTCT) was the 5' sense primer with nonhomologous sequences coding for the 27 nucleotide sequence. The results of the two reactions were two products with a

region of homology for the 27 nucleotide inserted sequence.

In the second step of the PCR technique, the templates with homologous regions were combined and allowed to go through denaturation, annealing, and extension to extend the template to completion. The only product that should extend under these conditions was the 5' PCR product sense-strand from the first PCR reaction annealing its 3' end with the 3' end of the 3' antisense PCR product from the second reaction. A1kPRIM1 and A1kPRIM2 were then added to the product which serves as the template for the PCR amplification. The PCR product was inserted into a pIC vector and was called pICA1k.6. This results in a full length product with the inserted 27 nucleotide DNA. The sequence of the insert in pICA1k.6b was determined by dideoxy termination sequencing incorporating ^{35}S labelled dATP.

The 0.6 kb insert in the clone of pICA1k.6 was inserted into the BamH I site in pICA1k.2 to generate a replication of the VJ_k, resulting in the construct pICA1k.8. Orientation was determined by a Bgl II + BamH I primary digest, and a Bgl II secondary digest. The 3.8 kb BamH I + Bgl II insert of pICA1k.8 coding for the two VJ_k genes was inserted into pH556, which is a vector carrying a kanamycin resistance gene. The insert was inserted into the EcoR I site of CIP pH556/EcoR I to form the construct pHA1k.1.

The 3.8 kb BamH I + Bgl II insert from pHA1k.1 was inserted into the BamH I site of a CIP treated pGk.5 mammalian expression vector, and orientation was confirmed by a BamH I + Cla I digest. This construct was termed pGkA1.13 and encoded the duplicated L'V exon but not the promoter and leader and had a 27 nucleotide linker between the L' and V regions in the exon. Ten μg of each of the DNAs pGkA1.13/Not1 and pNγ1A2.2/EcoRI were transfected into Ag8.653 mouse myeloma cells. After three weeks culture supernatants were screened for

binding to anti-IgG_1 polysera and GBS polysaccharide. Those binding most strongly were analyzed in the urea-PAGE system described above and the presence of oligomer confirmed.

EXAMPLE VII

In Vitro Characterization of Increased Binding Avidity

The higher molecular weight antibody had higher binding ability, or avidity, than did the 150kD form, as shown in Fig. 13. In this experiment, GBS strain I334 was bound to microtiter wells using poly-L-lysine. 1B1 antibody was fractionated by gel filtration on a Sephacryl® S-300 column to enrich for the higher molecular weight form. Equivalent protein concentrations of a transfectoma derived IgM antibody of the same binding specificity (t4B9), and the lower molecular weight form and the higher molecular forms of 1B1 were reacted with the GBS. Binding was assayed with horseradish peroxidase labeled anti-human mu-chain and gamma-chain specific antibodies.

The multivalent property of 1B1 was determined in an antibody capture assay. In Fig. 14A and Fig. 14B, microtiter plates were coated with an anti-idiotypic antibody which reacted with IgM t4B9, the IgG monomer, and the IgG multimer. In parallel tests, the IgM t4B9 (Fig. 14A), and low molecular weight fraction versus the high molecular weight-enriched fractions of 1B1 (Fig. 14B) were reacted to identical test wells. After washing, the ability of the bound antibody to further bind the same anti-idiotypic antibody, labeled with biotin for detection purposes, was determined. The high molecular weight-enriched fraction had additional binding

sites available for further anti-idiotype binding, similar to the IgM, whereas the low molecular weight fraction apparently had no additional sites available for binding. These data further suggest that the high molecular weight IgG antibody exists as an oligomer.

Further in vitro functional comparisons of monomeric and oligomeric IgG were performed. Although binding to antigen is usually obligatory for antibodies to mediate opsonic activity, binding activity alone does not predict whether an antibody will effectively enhance phagocytosis. Therefore the monoclonal antibodies were compared for their relative abilities to mediate opsonization and kill a GBS serotype III clinical isolate. The opsonophagocytic assays were performed essentially as described in Raff et al., supra, using strain IIIR (Bohnsack et al., J. Immunol. 143:3338 (1989). The studies compared the activity of 1B1 monomer and 1B1 dimer, and the IgM t4B9. The data, shown in Fig. 15, are reported as: Percent killed = 100 X (1 - [(CFU remaining after incubation with neutrophils, complement and test antibody)/(CFU remaining after incubation with neutrophils, complement and negative control antibody)]). By quantifying viable bacteria after each monoclonal antibody was incubated with the human neutrophils and human serum (complement), the dimer fraction showed 100-fold more activity than the monomer. When complement was omitted from the reaction mixture, none of the monoclonal antibodies were observed to demonstrate opsonic activity.

EXAMPLE VIII

Use of Oligomeric IgG for Treatment of Group B Streptococcal Infection in Neonatal Rats

Outbred Sprague-Dawley rat pups less than 48 hours old (housed with their mothers) were injected

intraperitoneally with 80-500 streptococci (varied among strains), 4 hours before having received 5-80 micrograms of either oligomer-enriched 1B1 antibody, monomeric 1B1 antibody, the t4B9 IgM antibody, or a control antibody to flagella of Pseudomonas aeruginosa. In all experiments, the rat pups were examined twice daily for symptoms and were scored for survival. The results of the experiments, summarized in Table II, demonstrated that the oligomer-enriched antibody protected significant numbers of animals from death at both low and high concentrations of antibody. In contrast, the monomeric IgG antibody only provided significant protection against one strain and then only at the highest antibody concentration.

TABLE II. Comparison of the Polymeric and Monomeric IgG1 Forms of the Anti-GBS MAb 4B9.

GBS Strain (Serotype)	Antibody	Dose (μg/rat) 80	40	20	5
I400 (Ic)	Polymeric	25/25[b]	-[c]	12/16[b]	5/9[b]
	Monomeric	19/49[a]	-	1/8	-
	IgM	-	-	24/26[b]	12/16[b]
	Negative Cont.	-	1/28	-	-
M94 (III)	Polymeric	-	4/7[a]	-	1/7
	Monomeric	1/6	-	0/7	-
	IgM	-	-	7/8[b]	4/7[a]
	Negative Cont.	-	0/7	-	-

[a] = $p < 0.05$

[b] = $p < 0.01$

c - = Not Done

EXAMPLE IX
Transplacental Passage of Oligomeric Antibody to Fetuses of Pregnant Rats

The ability of the high molecular weight oligomeric antibody to pass through the placenta and into the fetus, and thus into the subsequently delivered offspring, was compared between the monomeric and oligomeric antibody forms. An infant rat model was used as an animal model. Similar rat models have been used to predict the transplacental passage of antibody and other molecules to human fetuses. Brambell, Frontiers Biol. 18:234-276 (1970).

In one set of experiments, purified monomer, dimer (85% oligomer), and IgM monoclonal antibodies were injected intravenously into timed-pregnant Sprague-Dawley rats, at day 18-19 of gestation. On the day of, and every 2-3 days through 21 days after delivery, dams and pups were bled. The same dams were followed continuously, whereas pups could only be bled once before sacrifice. Therefore, half-lives in pups are calculated using different littermates at each sampling time. Human monoclonal antibody concentrations in rat sera were quantitated using an anti-human immunoglobulin ELISA as described in Example VII above, except that anti-human IgG from polyvalent sera was adsorbed to microtiter plate wells, and using an anti-group B polysaccharide antigen ELISA as described in Raff et al., supra. The anti-immunoglobulin assay detected monomer and oligomers equivalently and did not determine whether the oligomer was intact, while the antigen binding assay was 100-fold more sensitive for the oligomer than the monomer. By comparing the ratio of immunoglobulin concentrations between the two assays it was possible to establish what portion of the total immunoglobulin was oligomer. Antibody half-lives were calculated from the antigen binding ELISA data using a computer software program, as

described in Shumaker et al., Drug Metabol. Rev. 17:331 (1986). The results are shown in Table III.

Table III. Half-life of monoclonal antibody oligomer in rat dams and transplacentally passed to pups.

Rats	Antibody	Half-life (days)
Dams	Monomer	3
	Dimer	3
	IgM	1
Pups	Monomer	10
	Dimer	7
	IgM	ND*

*Not detectable, <1.0 ng/ml

In the dams the monomer and dimer each had half-lives of 3 days. In the pups the monomer and dimer half-lives were extended to 10 and 7 days, respectively. The IgM half-life was 1 day in the dams and was not transplacentally passed to the pups (below the lower detection limit of 1 ng/ml). Dimer concentrations found in the dams and pups on the day of delivery were 290 ± 120 ng/ml and 360 ± 120 ng/ml serum, respectively.

Another approach was used in other studies for determining transplacental passage. Two to three days prior to their anticipated delivery date, pregnant rats were injected intravenously with 246 μg of either low molecular weight IgG (monomer) (Dams 1 and 2) or oligomer (30%) IgG (Dams 3 and 4). Blood samples were collected from the dams two hours after antibody administration and on the day of delivery, and from the neonatal rats just after birth. Total human IgG and human IgG anti-group B Streptococcus antibody were determined in each blood sample using quantitative binding assays (ELISAs). By using anti-human IgG-specific enzyme-labeled secondary

antibodies, rat IgG was neither detected nor interfered with the quantitation of the injected human IgG.

Calculations of the amount of transplacentally passaged antibody were made as follows. When the low molecular weight IgG and the high molecular weight IgG were compared in the IgG quantitation assay, the high molecular weight-enriched IgG gave only a slightly higher signal per microgram IgG than an identical concentration of low molecular weight IgG (1.2:1.0, respectively). However, when the two antibodies were compared in the whole organism-binding ELISA, per microgram of IgG, the high molecular weight antibody had much greater binding activity than the low molecular antibody (3.5:1.0). Thus, on a per microgram basis the oligomer enriched IgG had approximately three to four fold more binding activity against group B streptococcal antigen than did the monomeric IgG. Therefore, the ratio of values for the IgG quantitation assay and the antigen binding assay for the transplacentally passed monomeric IgG should approximate 1.0. In contrast, the same calculations should result in ratios between the two assays approximating 3.5 for dams injected with high molecular weight-enriched IgG.

Data presented in Table IV show that in neonatal rats born to dams injected with monomeric IgG, the ratio of the IgG concentrations calculated by both assays roughly approximated 1.0 (average from Dam 1 pups was 0.89 and Dam 2 pups was 1.4). In comparison, the same ratio determined from sera of neonatal rats born to dams injected with predominately high molecular weight-enriched IgG approximated 3.0 (average from Dam 3 pups was 2.6 and Dam 4 was 3.6). These data agreed with the predictions made from the original standard curve generated using low molecular weight and high molecular weight-enriched IgG fractions. Thus, it may be concluded that the low molecular weight and high-molecular weight IgG antibodies were transplacentally passed with

approximately equal efficiency. In other experiments the parental IgM class antibody did not pass the placenta (data not presented). Therefore, the multivalent IgG monoclonal antibody should be useful when administered prophylactically to pregnant women at risk of delivering a baby with an increased likelihood of developing a life-threatening group B streptococcal infection.

TABLE IV

Transplacental Passage of Polymeric IgG In Maternal/Neonatal Rat Pairs

Dam#	IgG Injected[b]	Quantitation Method[c]	Dam/Neonate[a] (ug/ml IgG) Dam	1	2	3	4	Aver.[d]
1	Monomeric	IgG	5.8	15.9	13.2	12.6	15.0	
		Antigen	5.8	13.3	11.3	10.6	15.6	
		Ratio[e]	1.0	0.84	0.86	0.84	1.0	0.9
2	Monomeric	IgG	2.5	6.9	8.3	4.4	7.4	
		Antigen	2.3	10.6	10.6	5.9	11.2	
		Ratio	0.9	1.5	1.3	1.3	1.5	1.4
3	Polymeric	IgG	4.4	10.0	11.4	12.7	14.6	
		Antigen	15.5	20.9	36.6	23.6	44.8	
		Ratio	3.5	2.1	3.2	1.9	3.1	2.6
4	Polymeric	IgG	3.9	13.6	12.8	14.4	11.3	
		Antigen	18.1	48.0	38.3	50.7	48.4	
		Ratio	4.6	3.5	3.0	3.5	4.3	3.6

a = Dams were bled on day of delivery and neonates within hours after delivery
b = Each dam received 246 ug of either the monomeric or polymeric IgG antibody
c = IgG in serum samples was quantitated by IgG quantitation ELISA and by a Group B streptococcus antigen quantitation ELISA using purified monomeric IgG as the reference standard
d = Average of ratios from 4 neonates/dam
e = IgG concentration calculated by antigen ELISA / IgG concentration calculated by IgG ELISA

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A oligomeric IgG monoclonal antibody composition capable of binding antigen, the oligomeric antibody produced by a cell and comprising two or more immunoglobulin monomers of the same antigen binding specificity.

2. The oligomeric monoclonal antibody of claim 1, having two immunoglobulin monomers.

3. The oligomeric monoclonal antibody of claim 1, having three immunoglobulin monomers.

4. The oligomeric monoclonal antibody of claim 1, wherein the monomers are associated noncovalently.

5. The oligomeric monoclonal antibody of claim 1, wherein a light chain of at least one of the monomers contains an insert of a sequence of amino acids sufficient to cause polymerization.

6. The oligomeric monoclonal antibody of claim 5, wherein the insert is a sequence of amino acids comprising a light chain variable region.

7. The oligomeric monoclonal antibody of claim 5, wherein the insert is a sequence of amino acids comprising a substantial duplication of the light chain variable region.

8. The oligomeric monoclonal antibody of claim 7, wherein the duplication comprises the entire light chain variable region.

9. The oligomeric monoclonal antibody of claim 7, wherein both light chains of at least one of the monomers contain substantially duplicated light chain variable regions.

10. The oligomeric monoclonal antibody of claim 7, wherein the duplicated light chain variable region is inserted between the light chain variable region and the light chain constant region.

11. The oligomeric monoclonal antibody of claim 10, wherein the duplicated light chain variable region further includes a duplicated variable region signal sequence.

12. The oligomeric monoclonal antibody of claim 7, wherein an amino acid linker sequence is inserted at the N-terminus of the duplicated light chain variable region.

13. The oligomeric monoclonal antibody of claim 1, wherein the immunoglobulin heavy chain constant region is of the human gamma-$_1$ subclass.

14. The oligomeric monoclonal antibody of claim 1, wherein the immunoglobulin heavy chain constant region is of the human gamma-$_2$ subclass.

15. The oligomeric monoclonal antibody of claim 1, wherein the immunoglobulin monomers have a kappa chain.

16. The oligomeric monoclonal antibody of claim 15, wherein the kappa chain is substantially human.

17. The oligomeric monoclonal antibody composition of claim 1, which is able to cross the placenta into fetal circulation.

18. The oligomeric monoclonal antibody of claim 1, wherein immunoglobulin heavy and light chain variable regions are encoded by genes isolated from a cell line different from the source of the heavy chain constant region gene.

19. The oligomeric monoclonal antibody of claim 18, wherein the immunoglobulin variable region genes are isolated from a cell line that expresses an IgM antibody.

20. The oligomeric monoclonal antibody of claim 18, wherein the immunoglobulin light chain gene is isolated from a cell line expressing an IgM antibody.

21. The oligomeric monoclonal antibody of claim 1, wherein monomer light and heavy chain constant regions are substantially human.

22. The oligomeric monoclonal antibody of claim 21, wherein the monomer light and heavy chain variable regions are substantially human.

23. An oligomeric IgG monoclonal antibody composition capable of binding to group B streptococci, the oligomeric antibody being produced by a cell and comprising two or more immunoglobulin monomers of the same antigen binding specificity.

24. The oligomeric monoclonal antibody of claim 23, wherein the antibody is protective in vivo against infection by the streptococci.

25. A mammalian cell line which secretes a oligomeric IgG monoclonal antibody capable of binding antigen, said oligomeric antibody comprising two or more immunoglobulin monomers of the same antigen binding specificity.

26. The cell line of claim 25, which also secretes a monomeric antibody of the same antigen binding specificity as the oligomeric antibody.

27. The cell line of claim 26, wherein the monomeric antibody constitutes at least about 50% of the antibody secreted by the cell line.

28. The cell line of claim 27, wherein the oligomeric antibody comprises about 10-15% of the antibody secreted.

29. The cell line of claim 26, wherein one or both of the immunoglobulin light chains of monomeric immunoglobulins which combine to form oligomeric antibodies have a substantially duplicated variable region.

30. The cell line of claim 29, wherein substantially the entire variable region of the light chains is duplicated.

31. A cell line producing an antibody having substantially the characteristics of ATCC No. CRL 10293.

32. The cell line of claim 31, which is ATCC No. CRL 10293.

33. The oligomeric IgG monoclonal antibody produced by the cell line of claim 31.

34. A method for producing an oligomeric IgG monoclonal antibody specific for a selected antigen, which method comprises:

transfecting into host cells which express a gamma heavy chain, operably linked light chain genes coding for substantially duplicated variable regions and a constant region, wherein said light chain and a gamma heavy chain expressed by the host cells assemble into oligomeric antibodies which bind the selected antigen;

cultivating said transfected host cells and identifying transfected cells which secrete said oligomeric IgG antibody.

35. The method according to claim 34, wherein genes which code for the gamma heavy chain are transfected into the host cell.

36. The method according to claim 34, wherein the gamma heavy chain is endogenous to the host cell.

37. The method according to claim 34, wherein the oligomeric IgG monoclonal antibody is substantially human.

38. A method of preventing group B streptococcal infection in a newborn mammal, which comprises administering to a mother prior to delivery of the newborn an oligomeric IgG monoclonal antibody composition which is able to pass through the placenta and into fetal circulation and protect against group B streptococcal infection.

39. A pharmaceutical composition which comprises an oligomeric IgG monoclonal antibody capable of binding antigen, the oligomeric antibody produced by a cell and comprising two or more immunoglobulin monomers

of the same antigen binding specificity, and a pharmaceutically acceptable carrier.

40. The pharmaceutical composition of claim 39, wherein the oligomeric IgG antibody binds to and protects against group B streptococci.

41. An isolated DNA sequence which encodes an immunoglobulin light chain having substantially duplicated variable regions.

42. A DNA sequence according to claim 41, which corresponds substantially to Seq. ID. No. 13.

43. An isolated DNA sequence which encodes the variable region of an immunoglobulin light chain which corresponds substantially to Seq. ID. No. 14.

44. An isolated DNA sequence which encodes the variable region of an immunoglobulin heavy chain variable region, the DNA sequence corresponding substantially to Seq. ID. No. 15.

45. A DNA construct comprising the following operably linked elements:

a) a transcriptional promoter;

b) a DNA sequence which encodes an immunoglobulin light chain having substantially duplicated variable regions; and

c) a transcriptional terminator.

46. The DNA construct of claim 45, wherein the DNA sequence corresponds substantially to Seq. ID. No. 13.

47. A cultured mammalian cell containing a DNA construct according to claim 45.

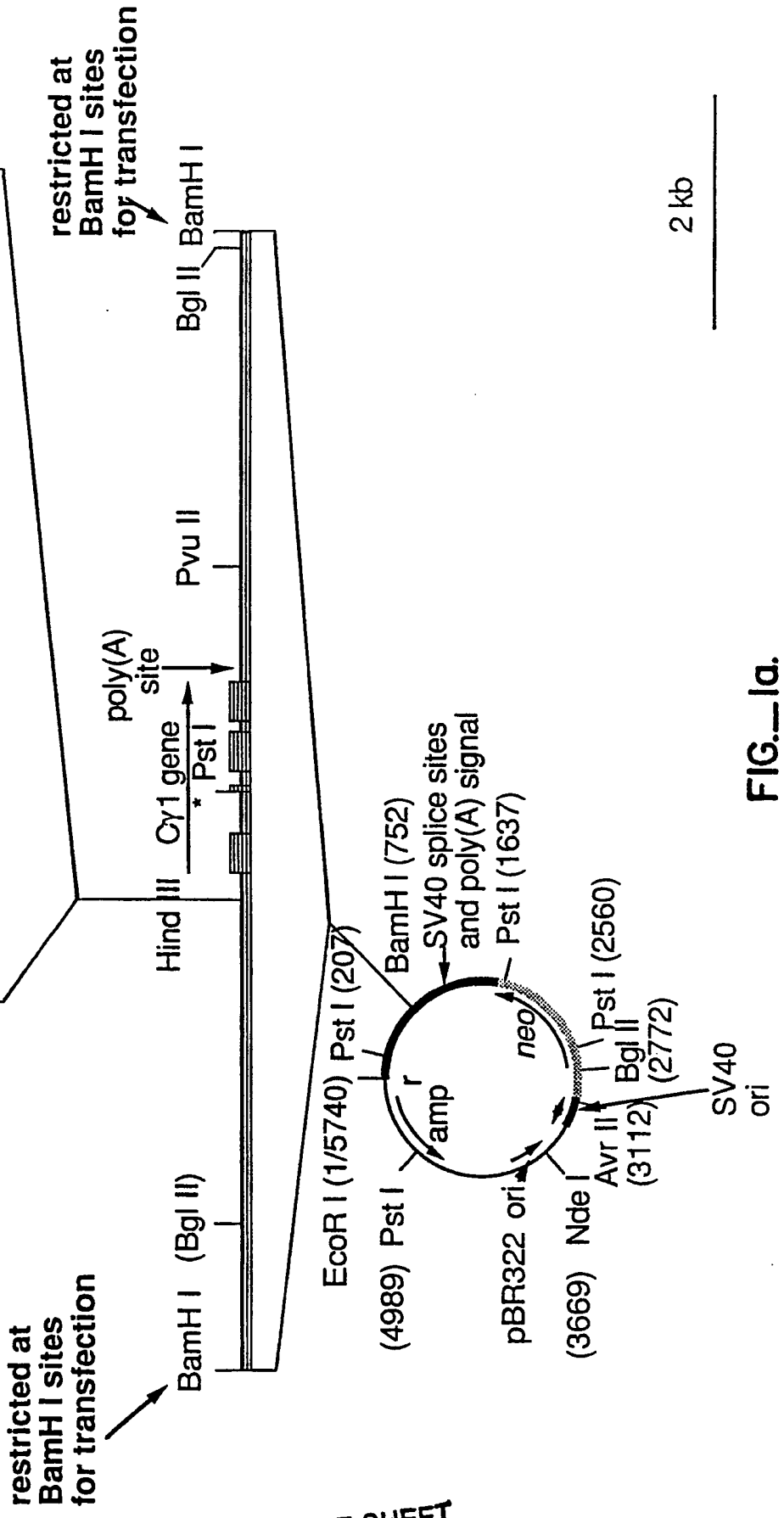

pNγ1A2.1
Hind III EcoR I
Bgl II
L-VDJ gene
Bgl II Hind III
el
restricted at BamH I sites for transfection
Bgl II BamH I
Pvu II
poly(A) site
Cγ1 gene
* Pst I
Hind III
BamH I (Bgl II)
restricted at BamH I sites for transfection
EcoR I (1/5740) Pst I (207)
BamH I (752)
SV40 splice sites and poly(A) signal
Pst I (1637)
(4989) Pst I
r
amp
neo
pBR322 ori
(3669) Nde I
Avr II (3112)
Bgl II (2772)
Pst I (2560)
SV40 ori
2 kb

FIG._1a.

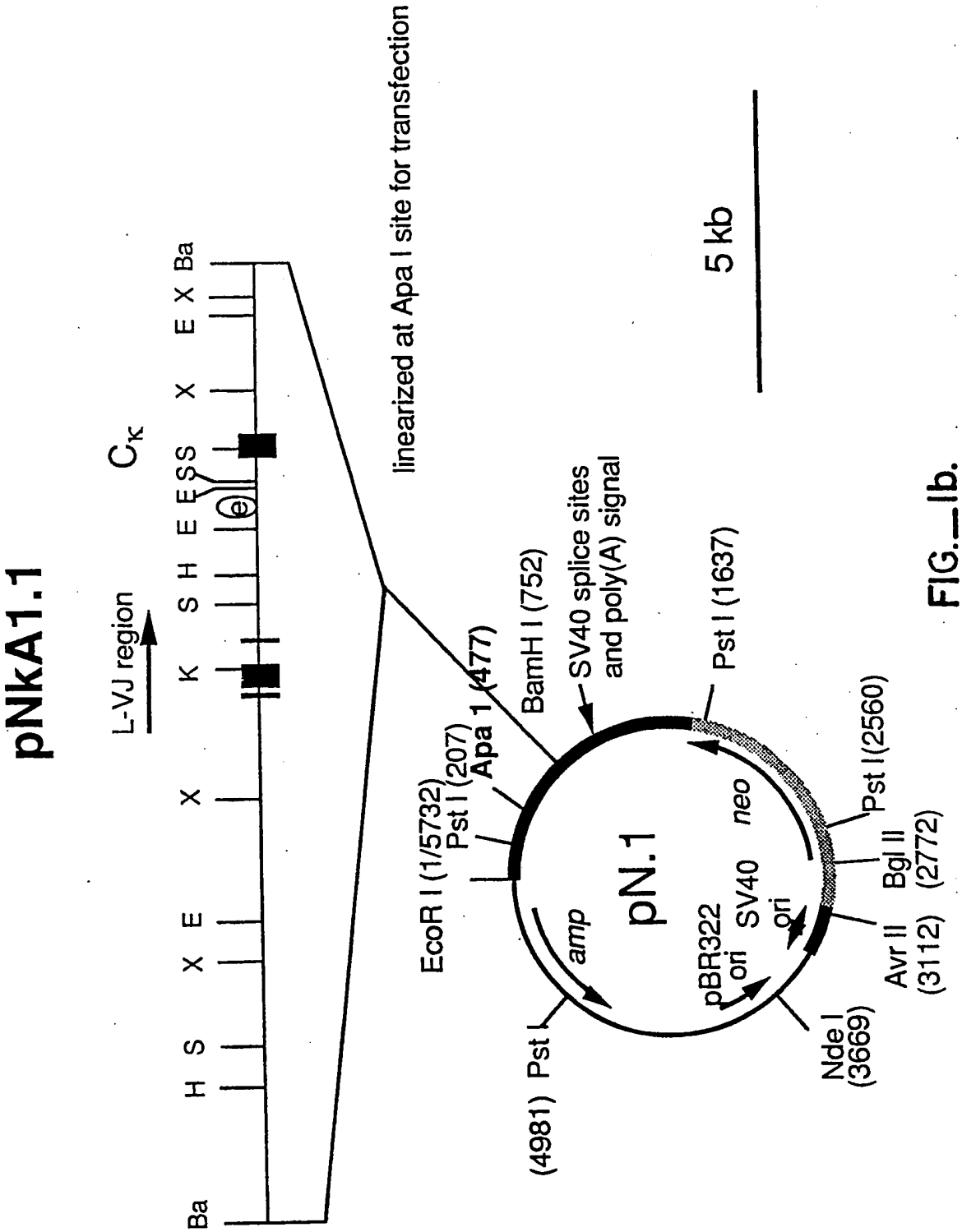

pNkA1.1
L-VJ region
C_κ
Ba H S X E X K S H E E S S X E X Ba
e
linearized at Apa I site for transfection
5 kb
EcoR I (1/5732)
Pst I (207)
Apa 1 (477)
BamH I (752)
SV40 splice sites
and poly(A) signal
Pst I (1637)
Pst I (2560)
Bgl II
(2772)
Avr II
(3112)
Nde I
(3669)
Pst I
(4981)
amp
pN.1
pBR322
ori
SV40
ori
neo

FIG._Ib.

Fig. 2A

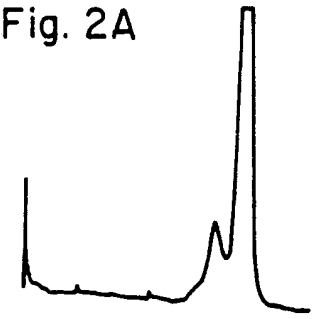


Fig. 2B

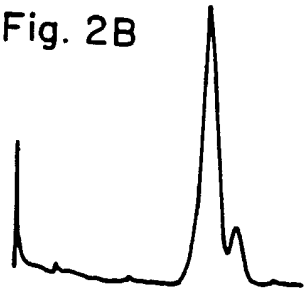
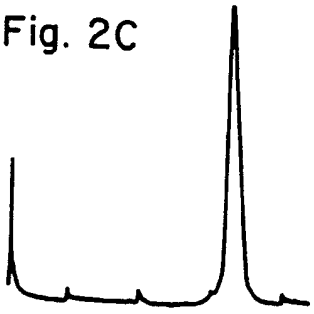


Fig. 2C



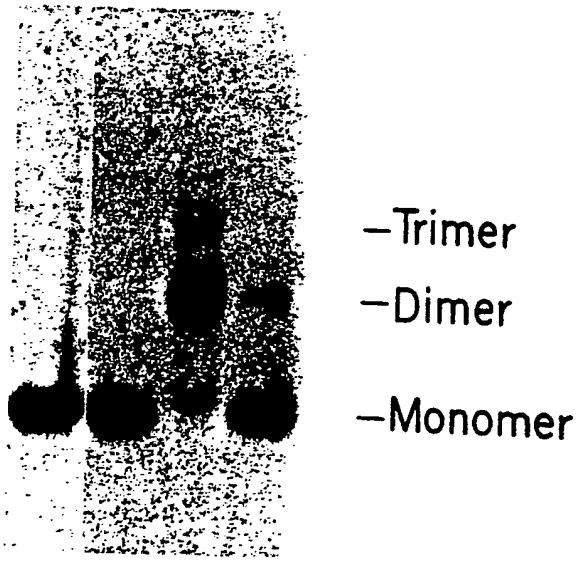


Fig. 3

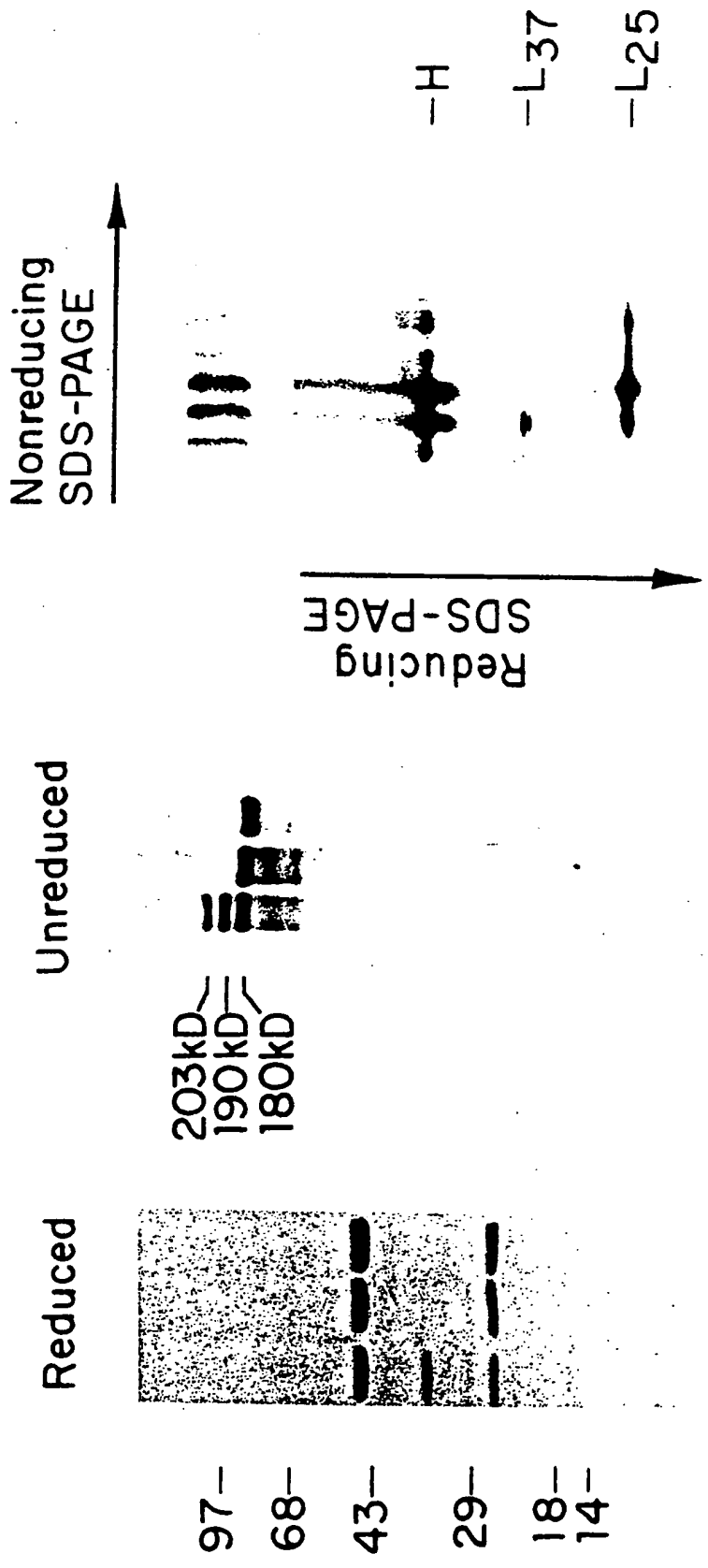


FIG_4C.

FIG_4B.

FIG_4A.

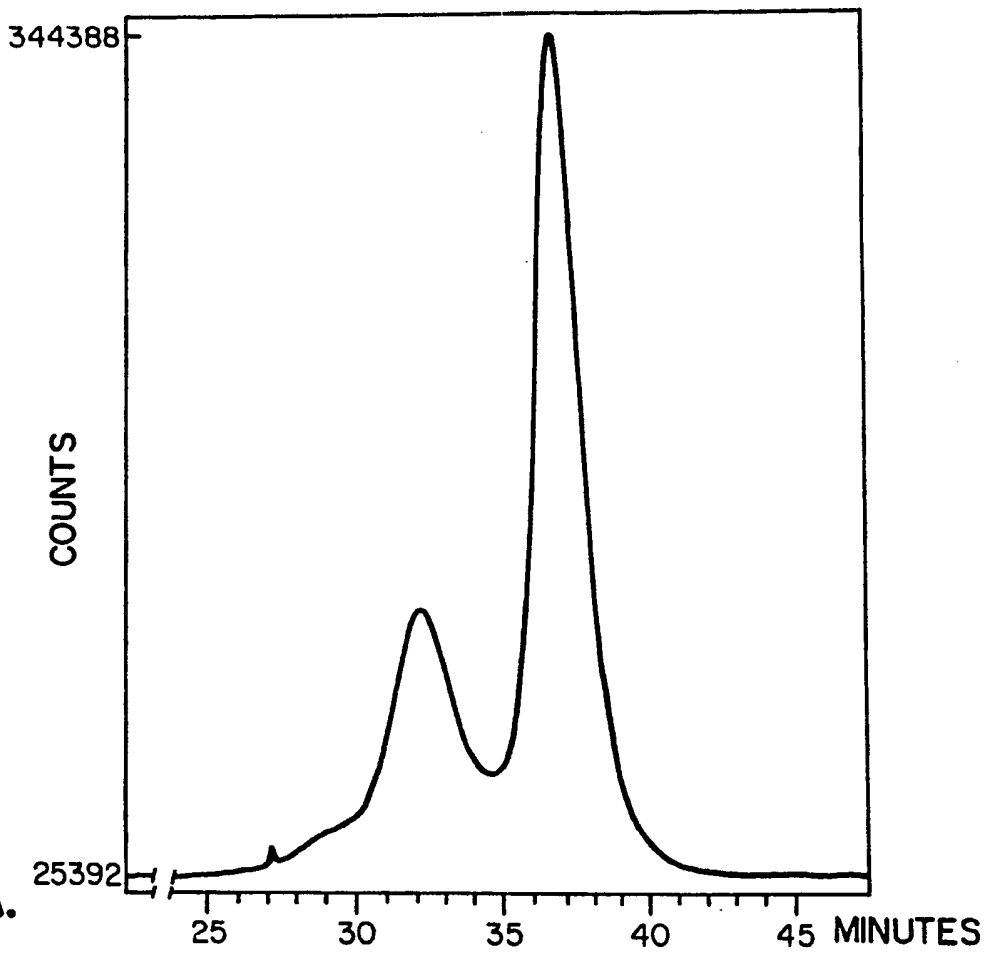

344388
25392
COUNTS
25
30
35
40
45
MINUTES
30
35
40
FRACTION NUMBER
(RETENTION TIME)

FIG._5A.

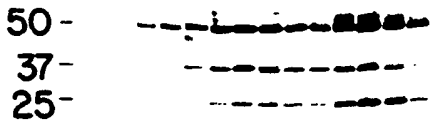

50
37
25

FIG._5B.

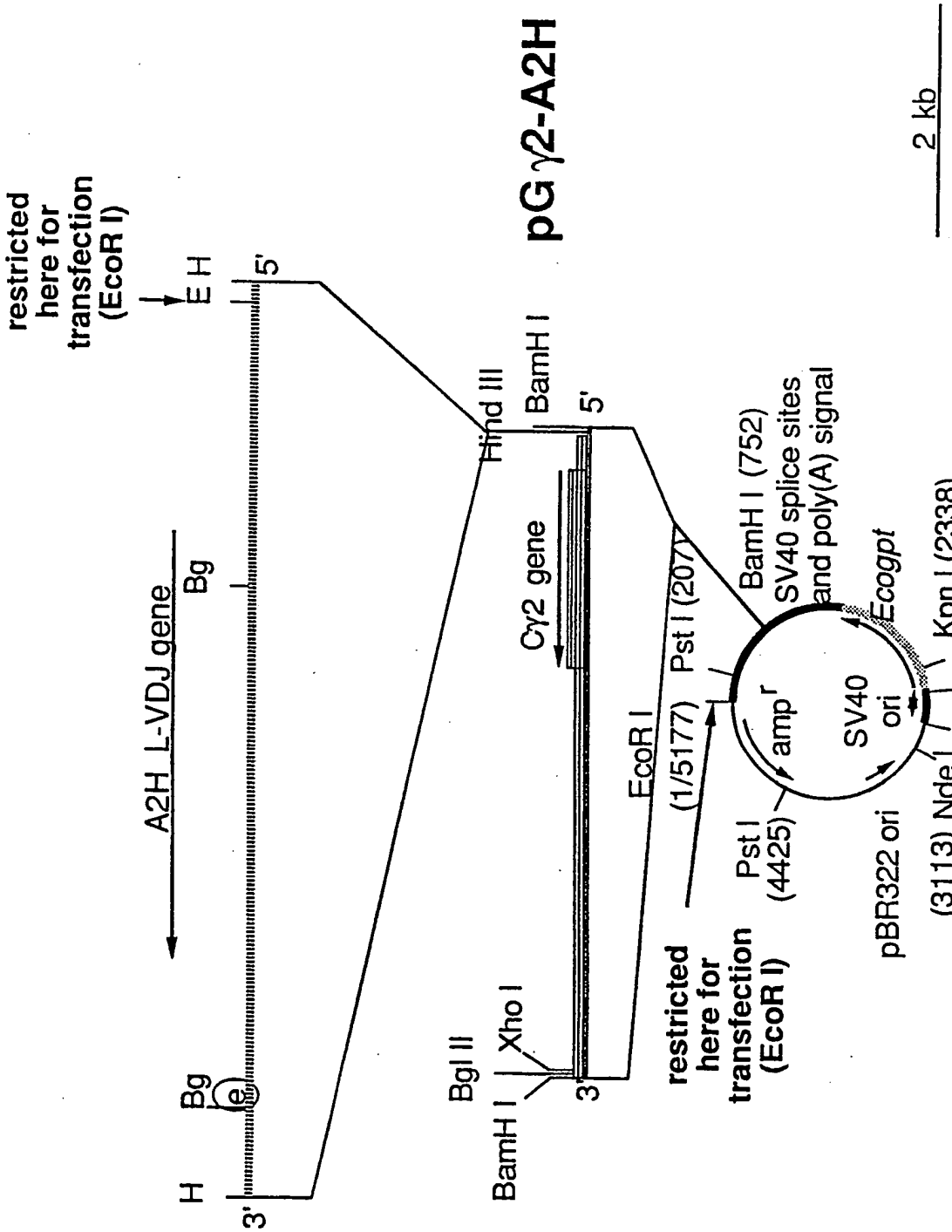

pGγ2-A2H
restricted here for transfection (EcoR I)
E H
5'
A2H L-VDJ gene
Bg
Bg
(e)
H
3'
Hind III
BamH I
5'
Cγ2 gene
Bgl II
Xho I
BamH I
3'
EcoR I
(1/5177)
Pst I (207)
restricted here for transfection (EcoR I)
Pst I (4425)
amp r
pBR322 ori
SV40 ori
BamH I (752)
SV40 splice sites and poly(A) signal
Ecogpt
(3113) Nde I
Pvu II (2882)
Kpn I (2338)
Avr II (2556)
2 kb

FIG._6.

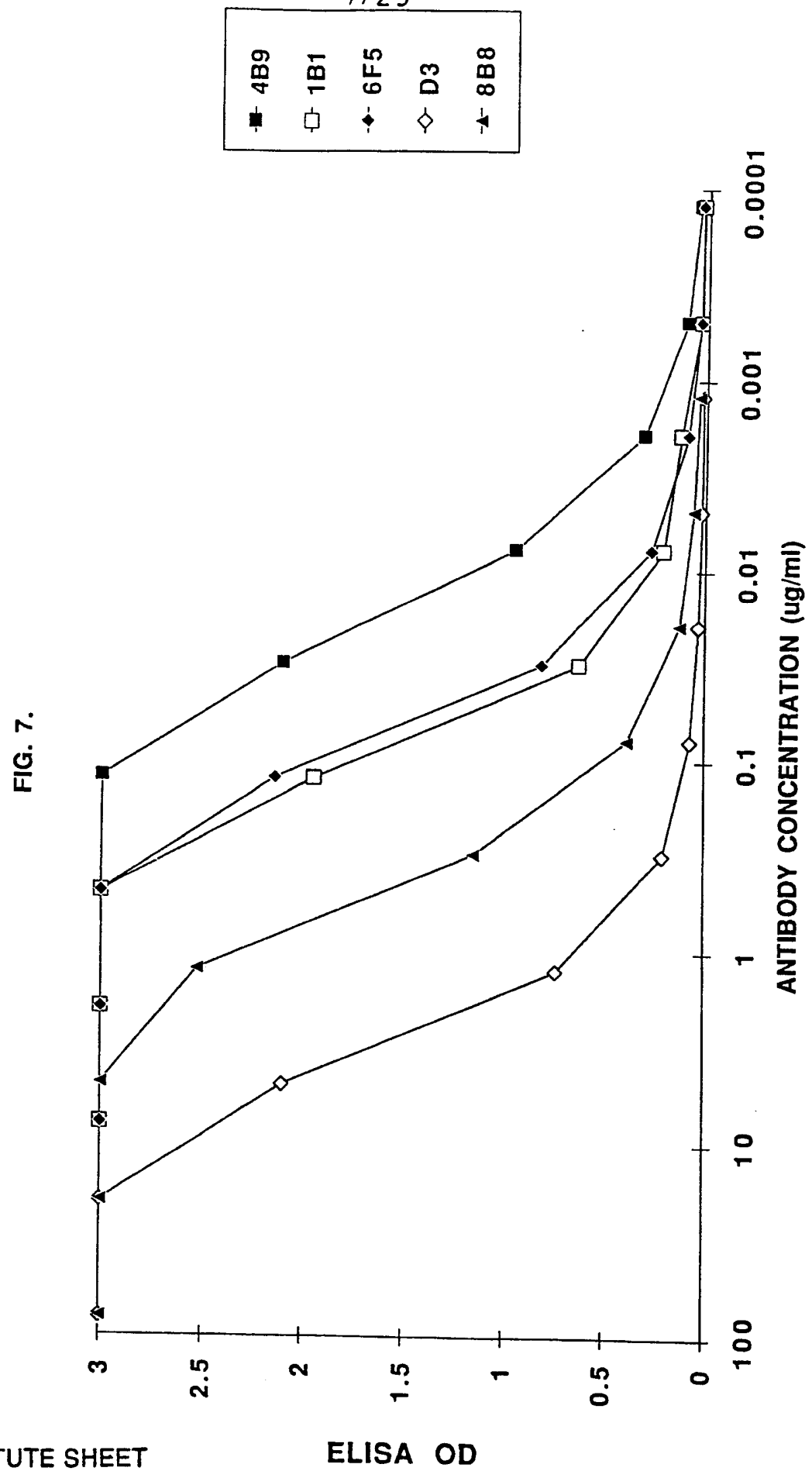

4B9
1B1
6F5
D3
8B8
ELISA OD
3
2.5
2
1.5
1
0.5
0
ANTIBODY CONCENTRATION (ug/ml)
100
10
1
0.1
0.01
0.001
0.0001

FIG. 7.

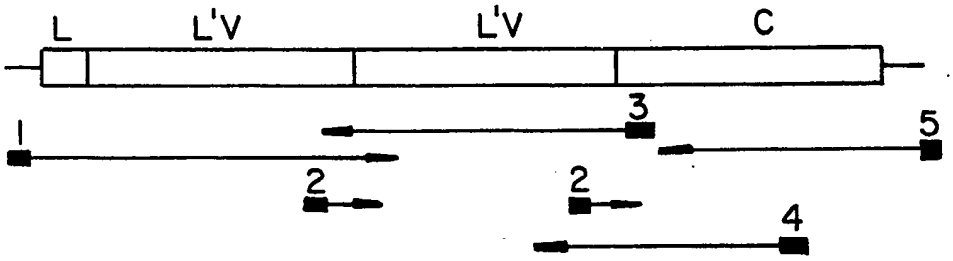


FIG._8.

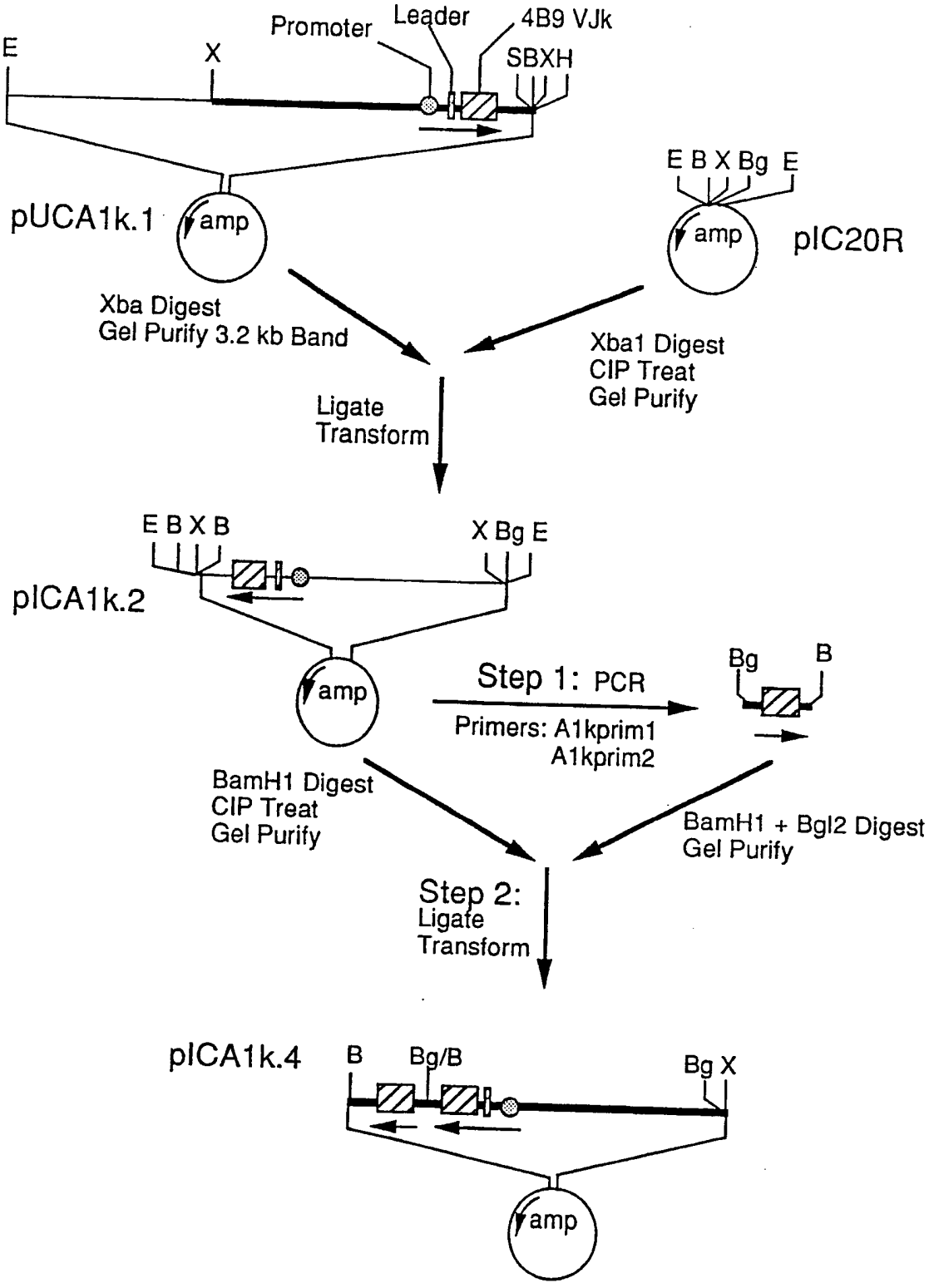
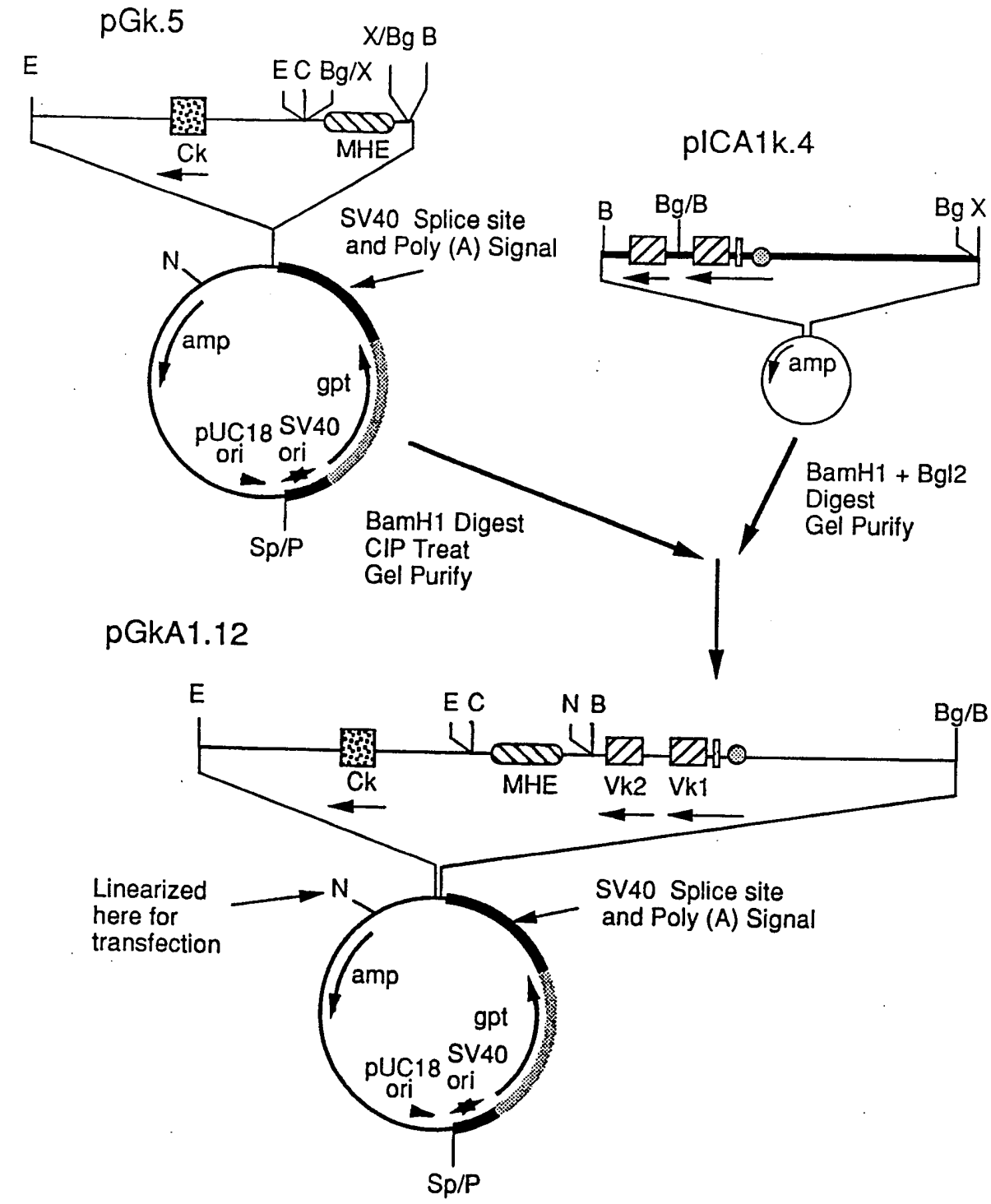

Promoter
Leader
4B9 VJk
E
X
SBXH
E B X Bg E
pUCA1k.1
amp
amp
pIC20R
Xba Digest
Gel Purify 3.2 kb Band
Xba1 Digest
CIP Treat
Gel Purify
Ligate
Transform
E B X B
X Bg E
pICA1k.2
amp
Step 1: PCR
Primers: A1kprim1
A1kprim2
Bg
B
BamH1 Digest
CIP Treat
Gel Purify
BamH1 + Bgl2 Digest
Gel Purify
Step 2:
Ligate
Transform
pICA1k.4
B
Bg/B
Bg X
amp

FIG._9.
(1 OF 2)



Symbols: E = EcoR1; X = Xba1; S = Sac1; H = Hind3; Bg = Bgl2; C = Cla1; N = Not1; Sp = Sph1; P = Pvu1

FIG._9.
(2 OF 2)

SUBSTITUTE SHEET

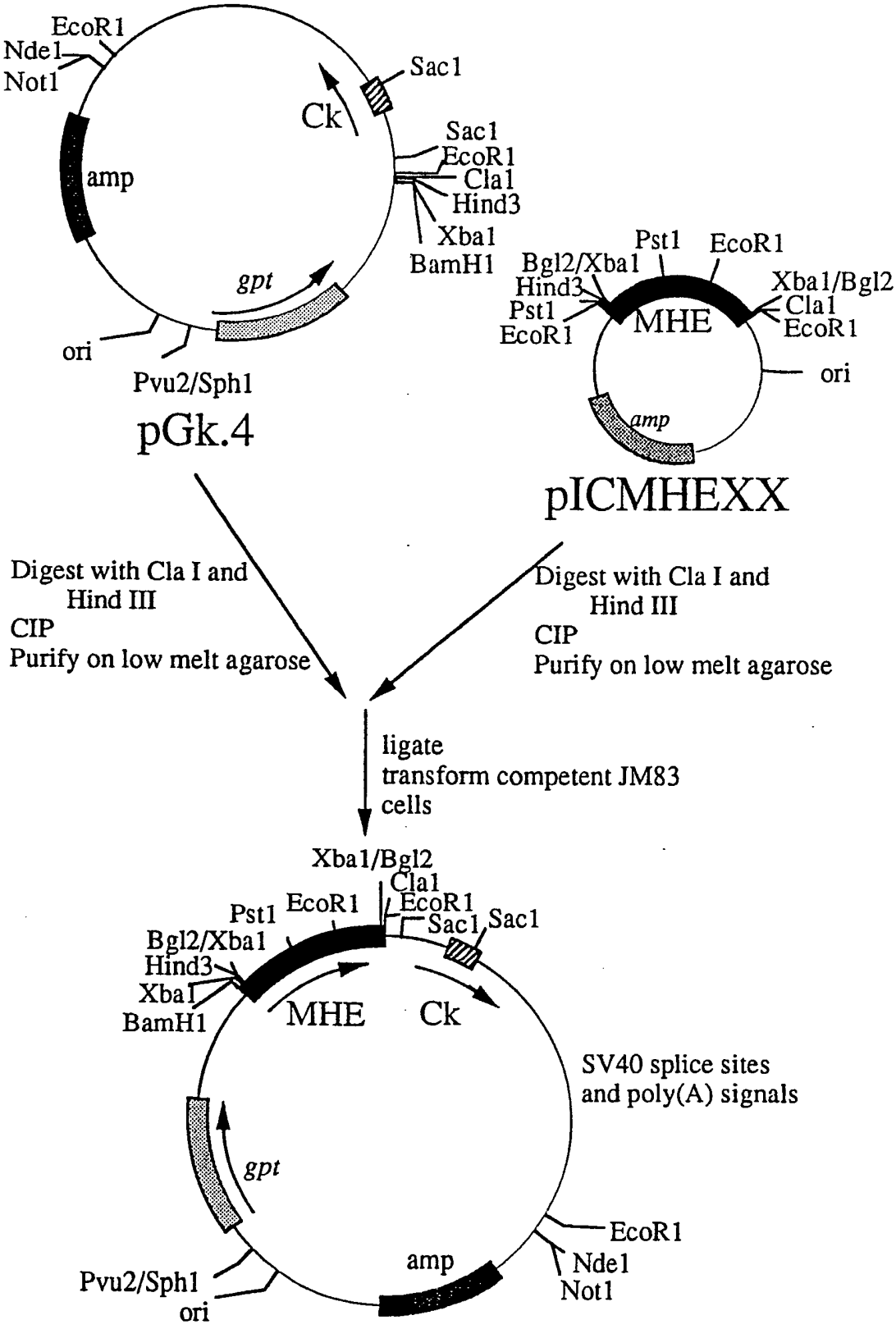

EcoR1
Nde1
Not1
Sac1
Ck
Sac1
EcoR1
Cla1
Hind3
Xba1
BamH1
amp
gpt
ori
Pvu2/Sph1
pGk.4
Pst1
EcoR1
Bgl2/Xba1
Hind3
Pst1
EcoR1
Xba1/Bgl2
Cla1
EcoR1
MHE
ori
amp
pICMHEXX
Digest with Cla I and
Hind III
CIP
Purify on low melt agarose
Digest with Cla I and
Hind III
CIP
Purify on low melt agarose
ligate
transform competent JM83
cells
Xba1/Bgl2
Cla1
Pst1
EcoR1
EcoR1
Sac1
Sac1
Bgl2/Xba1
Hind3
Xba1
BamH1
MHE
Ck
SV40 splice sites
and poly(A) signals
gpt
EcoR1
amp
Nde1
Not1
Pvu2/Sph1
ori
pGk.5

FIG._10.
(1 OF 4)

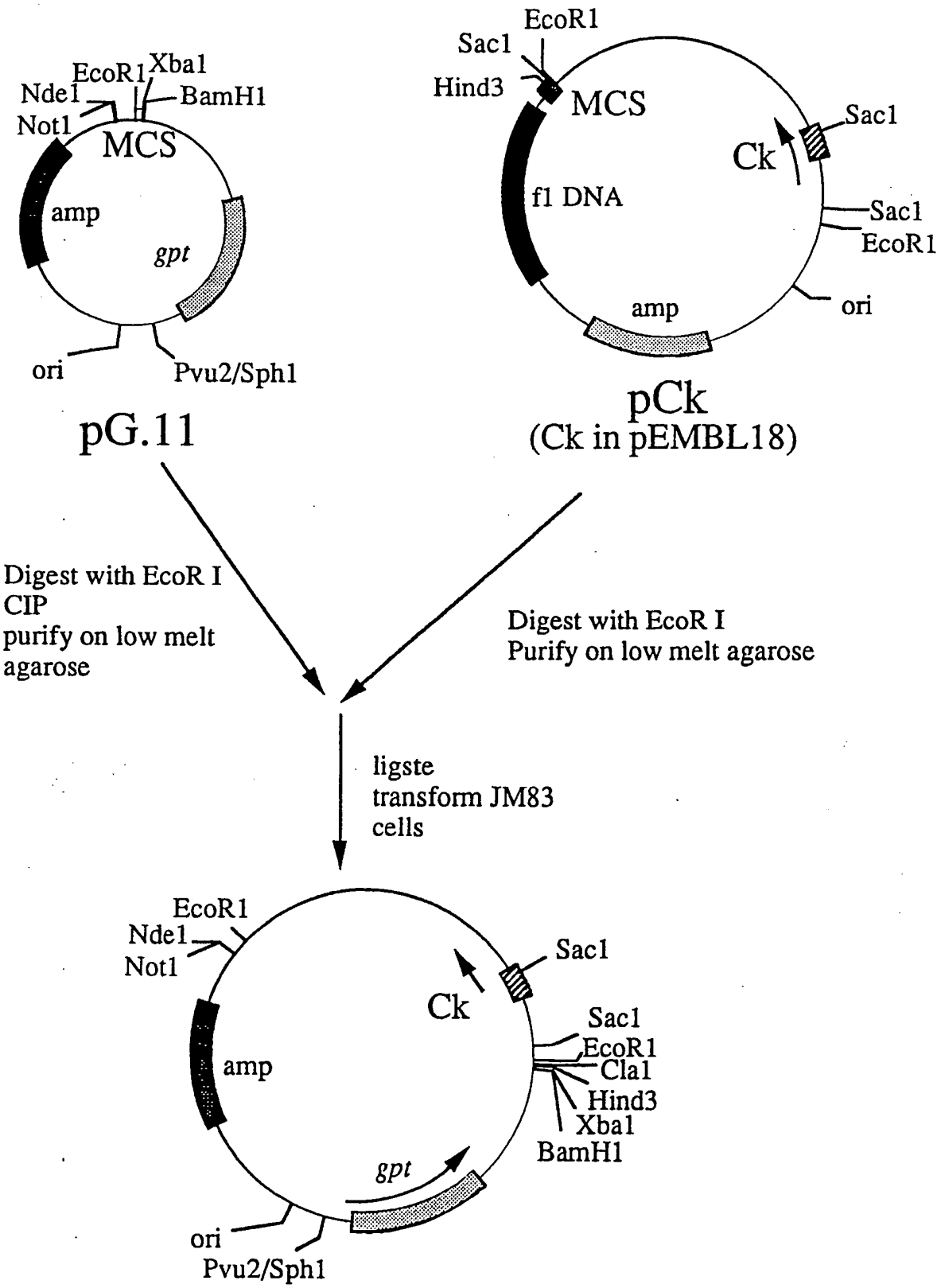

EcoR1
Xba1
Nde1
BamH1
Not1
MCS
amp
gpt
ori
Pvu2/Sph1
pG.11
EcoR1
Sac1
Hind3
MCS
Sac1
Ck
f1 DNA
Sac1
EcoR1
amp
ori
pCk
(Ck in pEMBL18)
Digest with EcoR I
CIP
purify on low melt
agarose
Digest with EcoR I
Purify on low melt agarose
ligste
transform JM83
cells
EcoR1
Nde1
Not1
Sac1
Ck
Sac1
EcoR1
Cla1
Hind3
Xba1
BamH1
amp
gpt
ori
Pvu2/Sph1
pGk.4

FIG._10.
(2 OF 4)

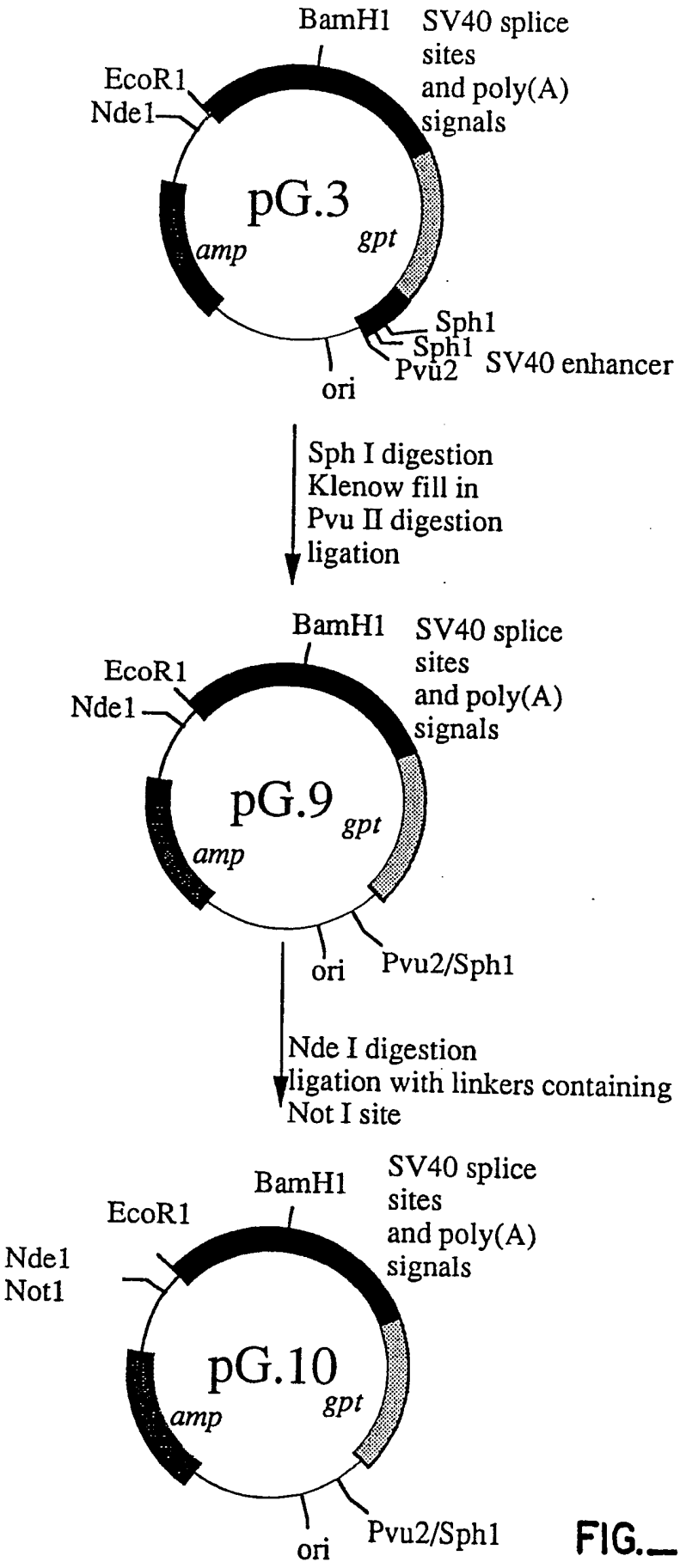

BamH1
SV40 splice sites and poly(A) signals
EcoR1
Nde1
pG.3
amp
gpt
Sph1
Sph1
Pvu2
SV40 enhancer
ori
Sph I digestion
Klenow fill in
Pvu II digestion
ligation
BamH1
SV40 splice sites and poly(A) signals
EcoR1
Nde1
pG.9
gpt
amp
ori
Pvu2/Sph1
Nde I digestion
ligation with linkers containing
Not I site
BamH1
SV40 splice sites and poly(A) signals
EcoR1
Nde1
Not1
pG.10
amp
gpt
ori
Pvu2/Sph1

FIG._10.

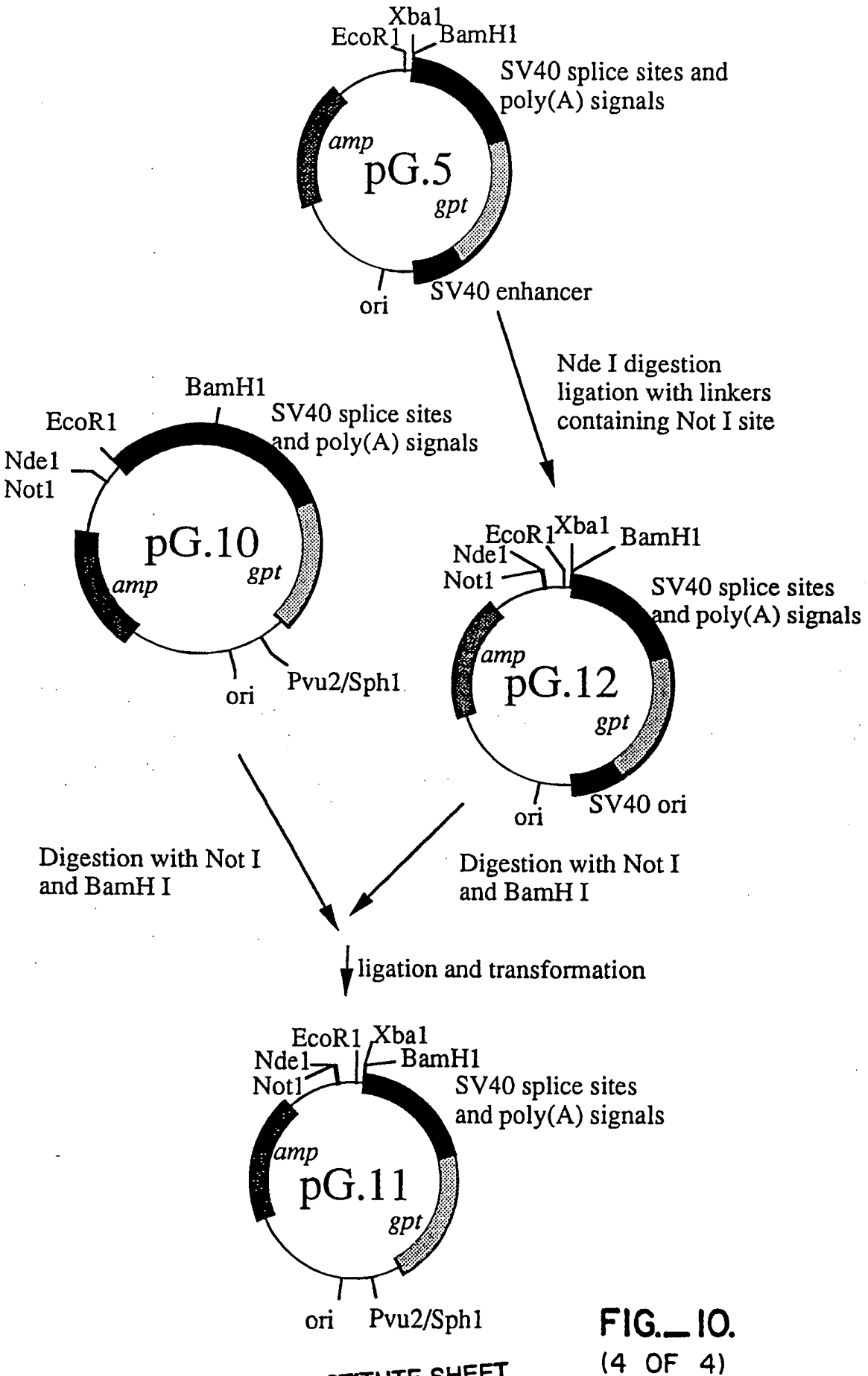


FIG._10.
(4 OF 4)

SUBSTITUTE SHEET



1 2 3 4

Fig. 11

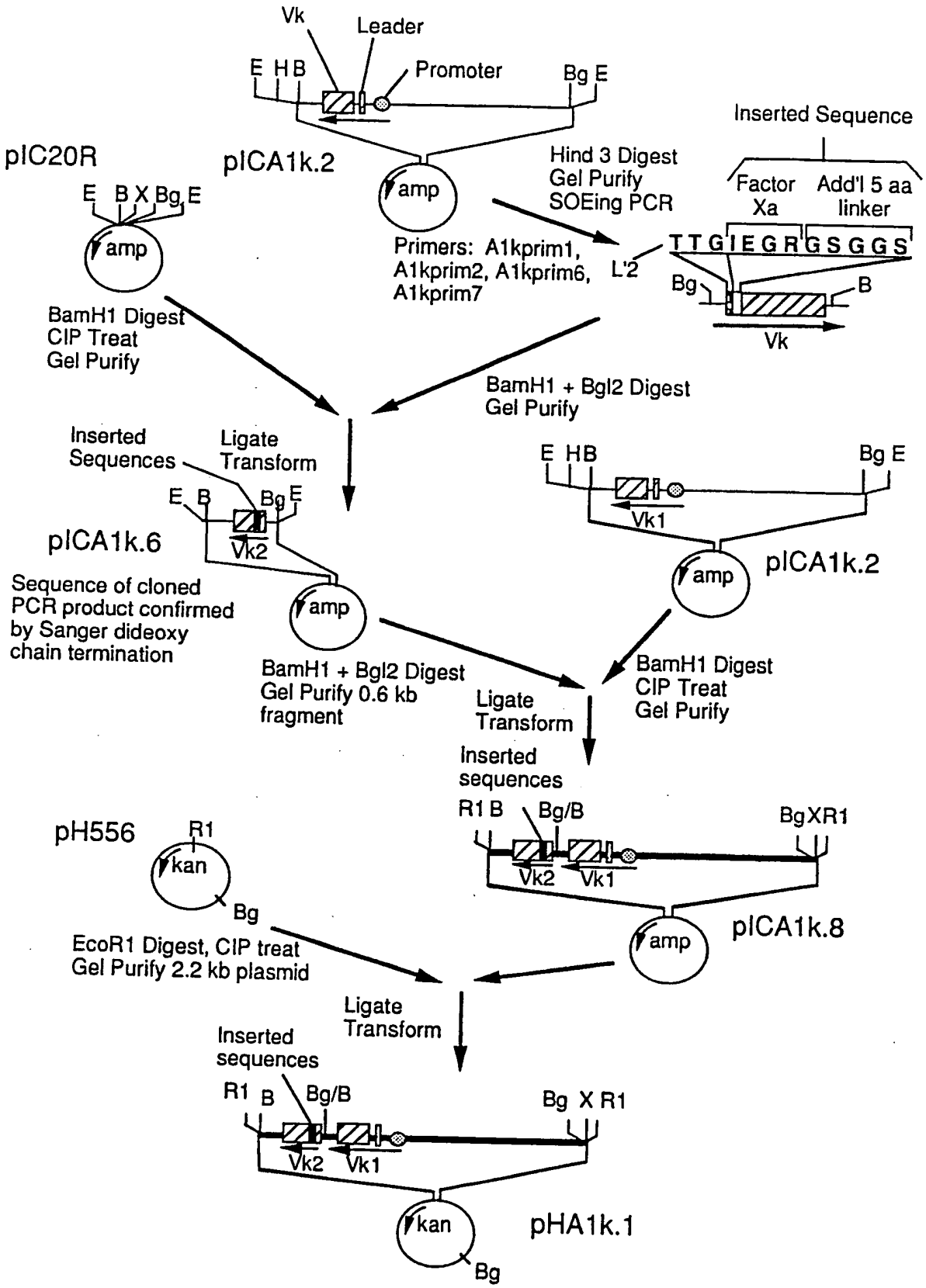
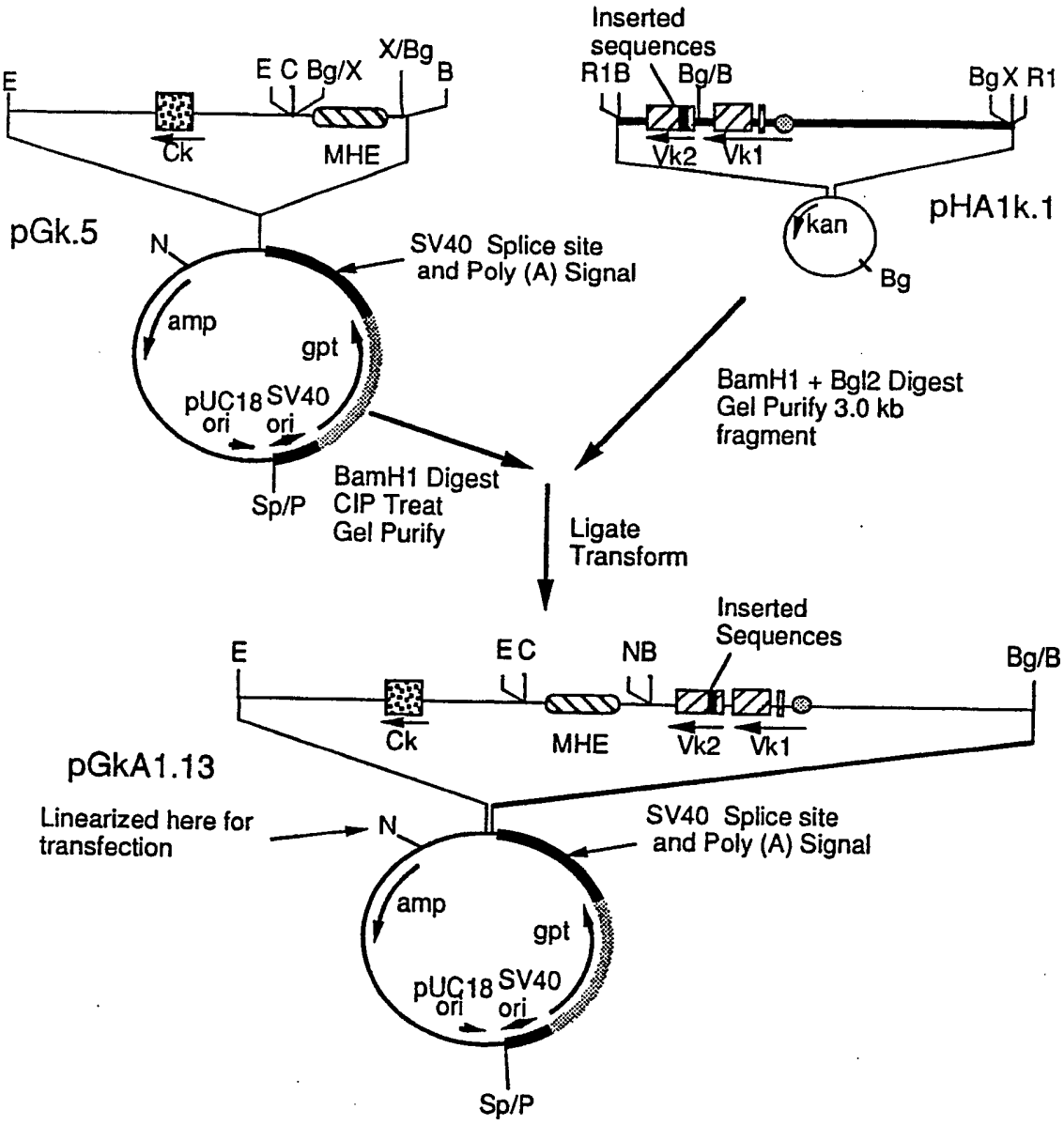


FIG._12.

(1 OF 2)

SUBSTITUTE SHEET



Symbols: E = EcoR1; X = Xba1; S = Sac1; H = Hind3;
Bg = Bgl2; C = Cla1; N = Not1; Sp = Sph1; P = Pvu2

FIG._12.

(2 OF 2)

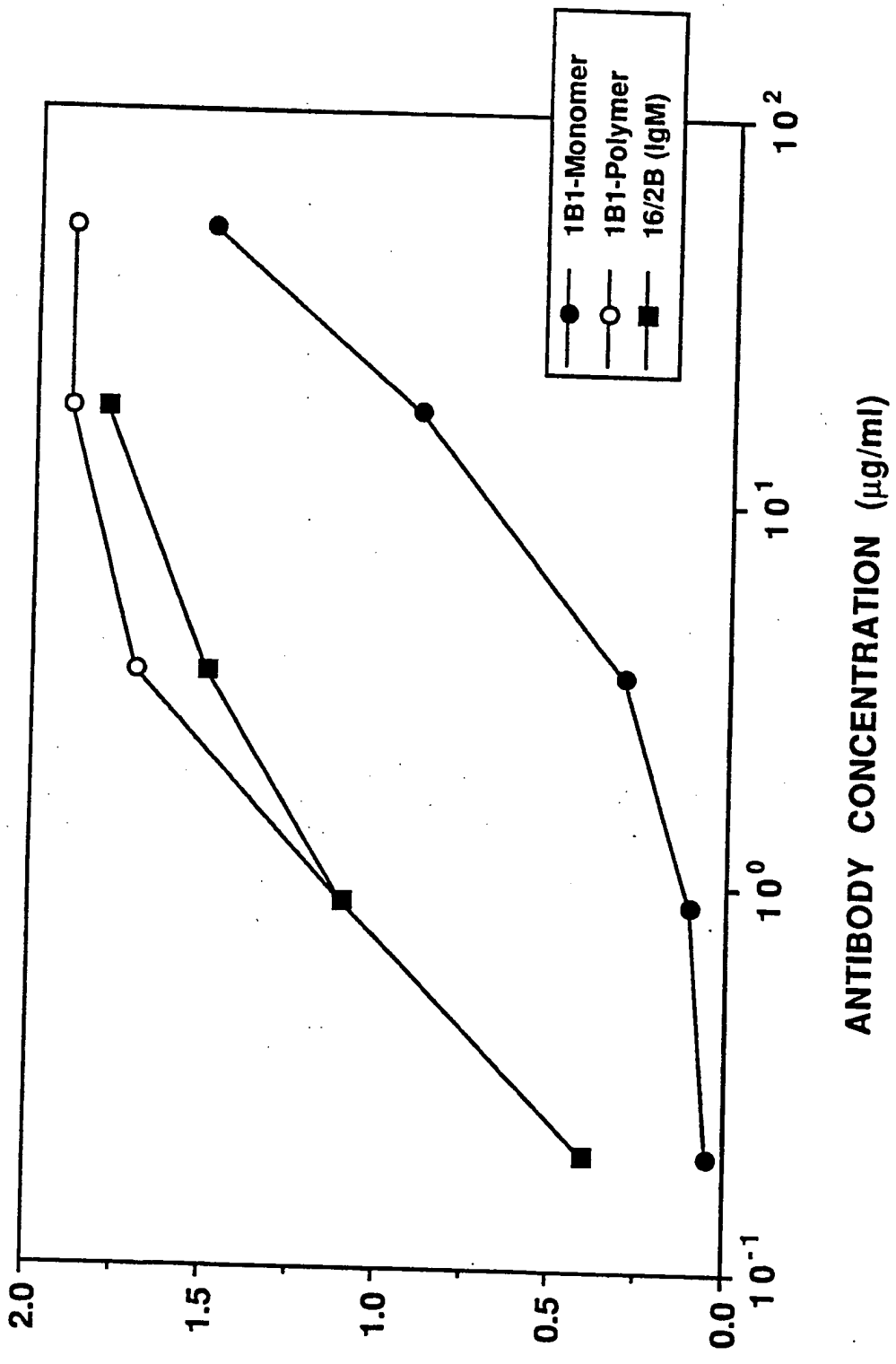

1B1-Monomer
1B1-Polymer
16/2B (IgM)
2.0
1.5
1.0
0.5
0.0
10^{-1}
10^{0}
10^{1}
10^{2}
ELISA OD
ANTIBODY CONCENTRATION (µg/ml)

Fig. 13

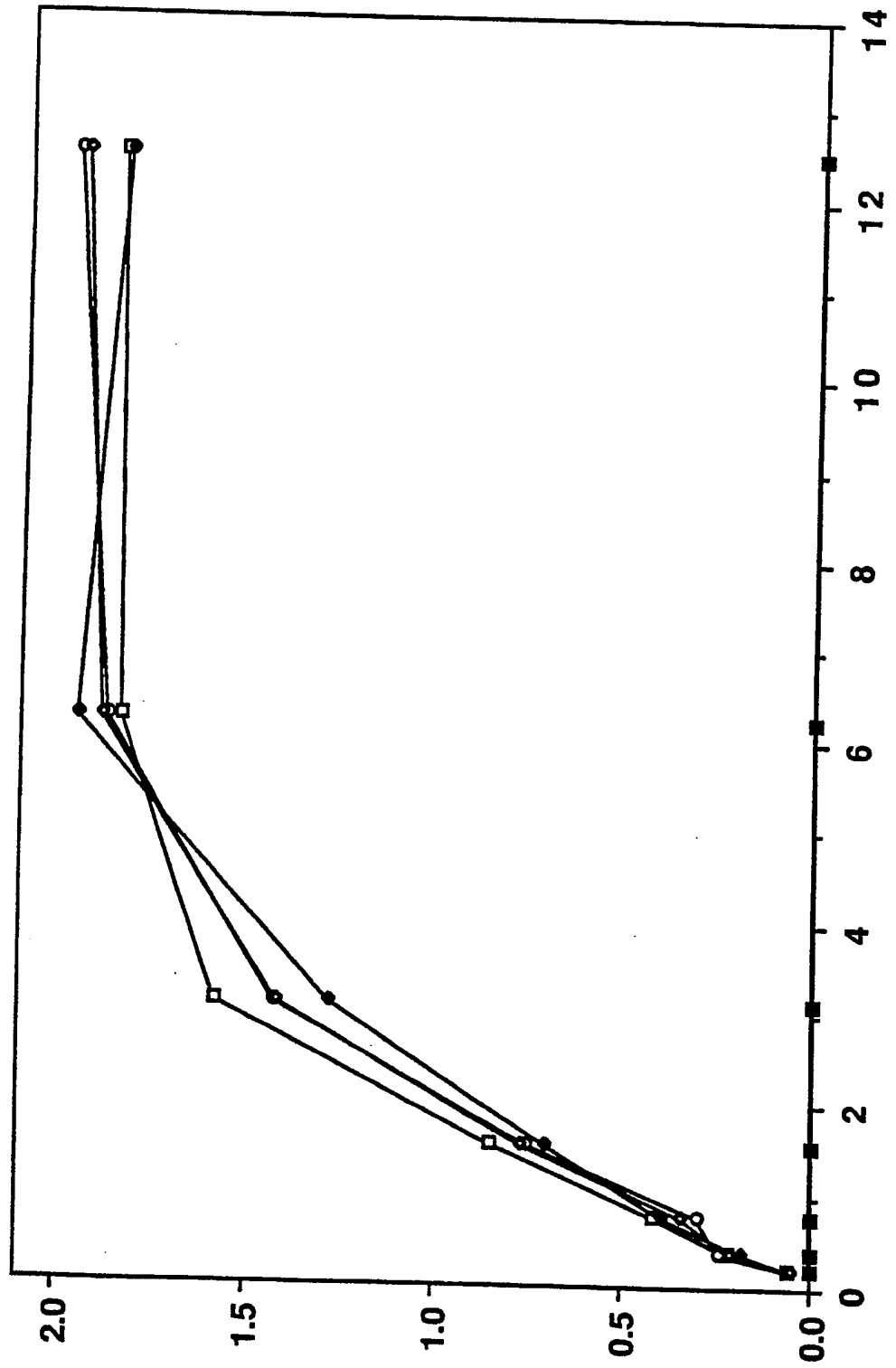


Fig. 14A

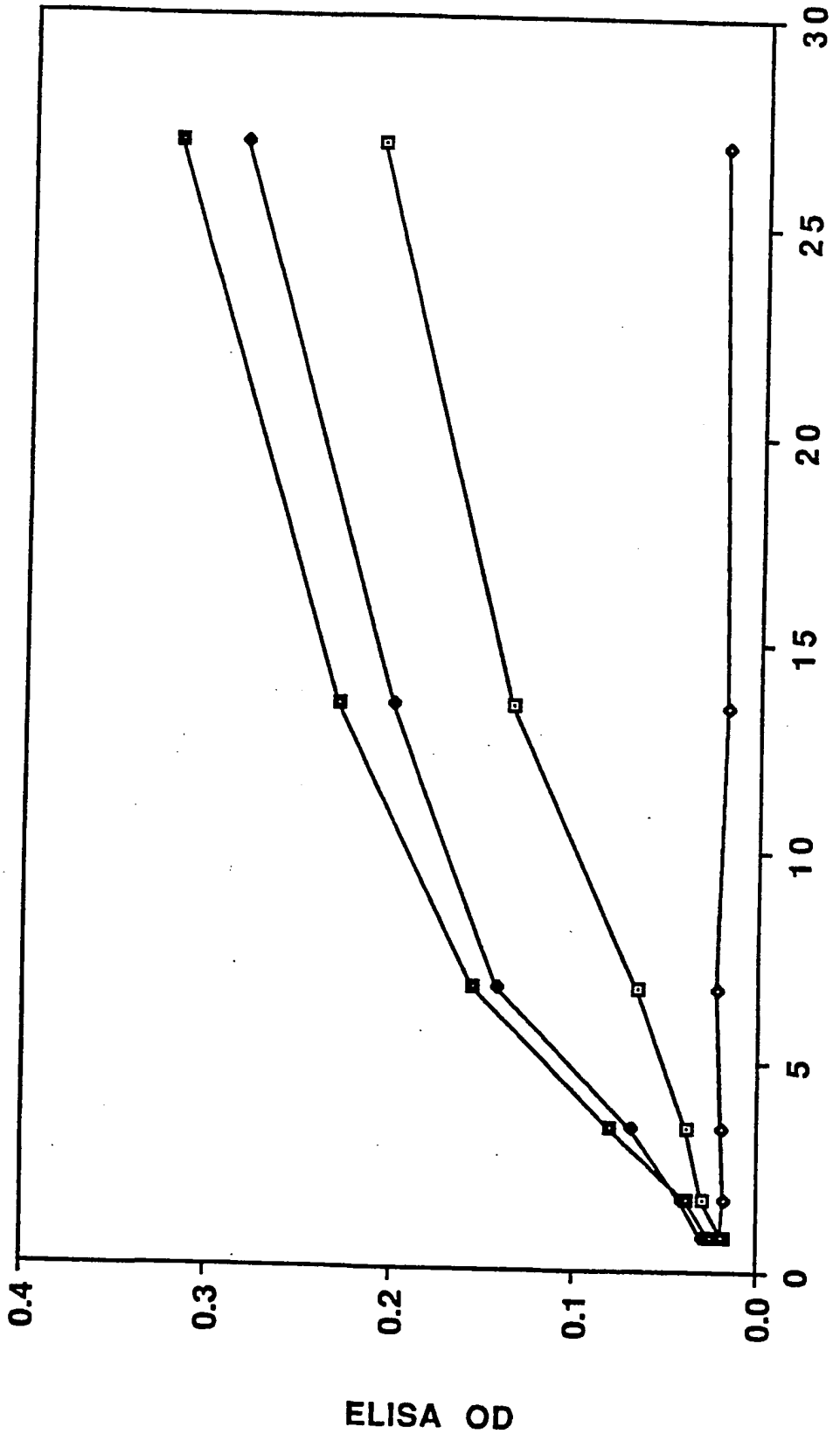


Fig. 14B

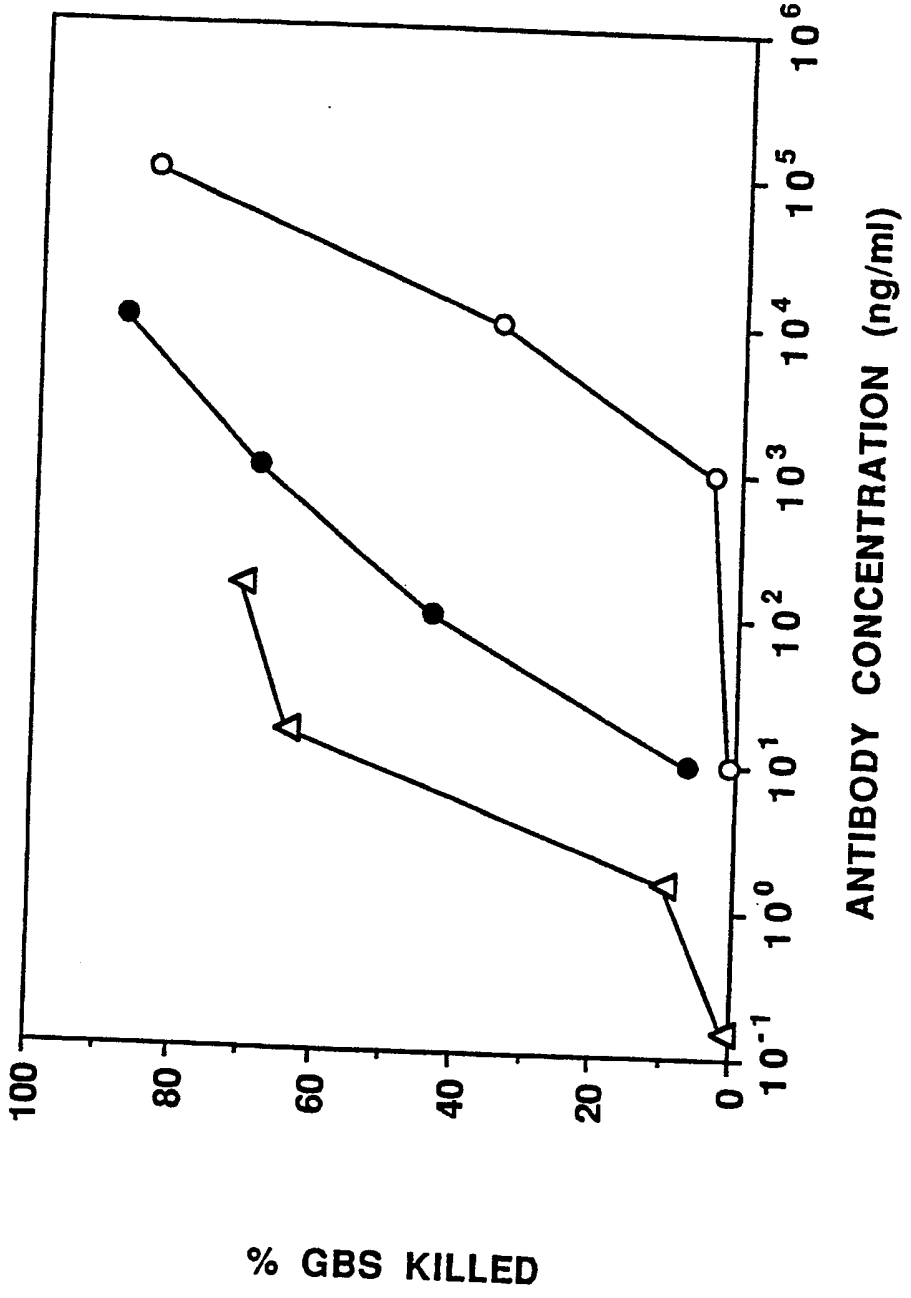

100
80
60
40
20
0
% GBS KILLED
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
ANTIBODY CONCENTRATION (ng/ml)

FIG. 15

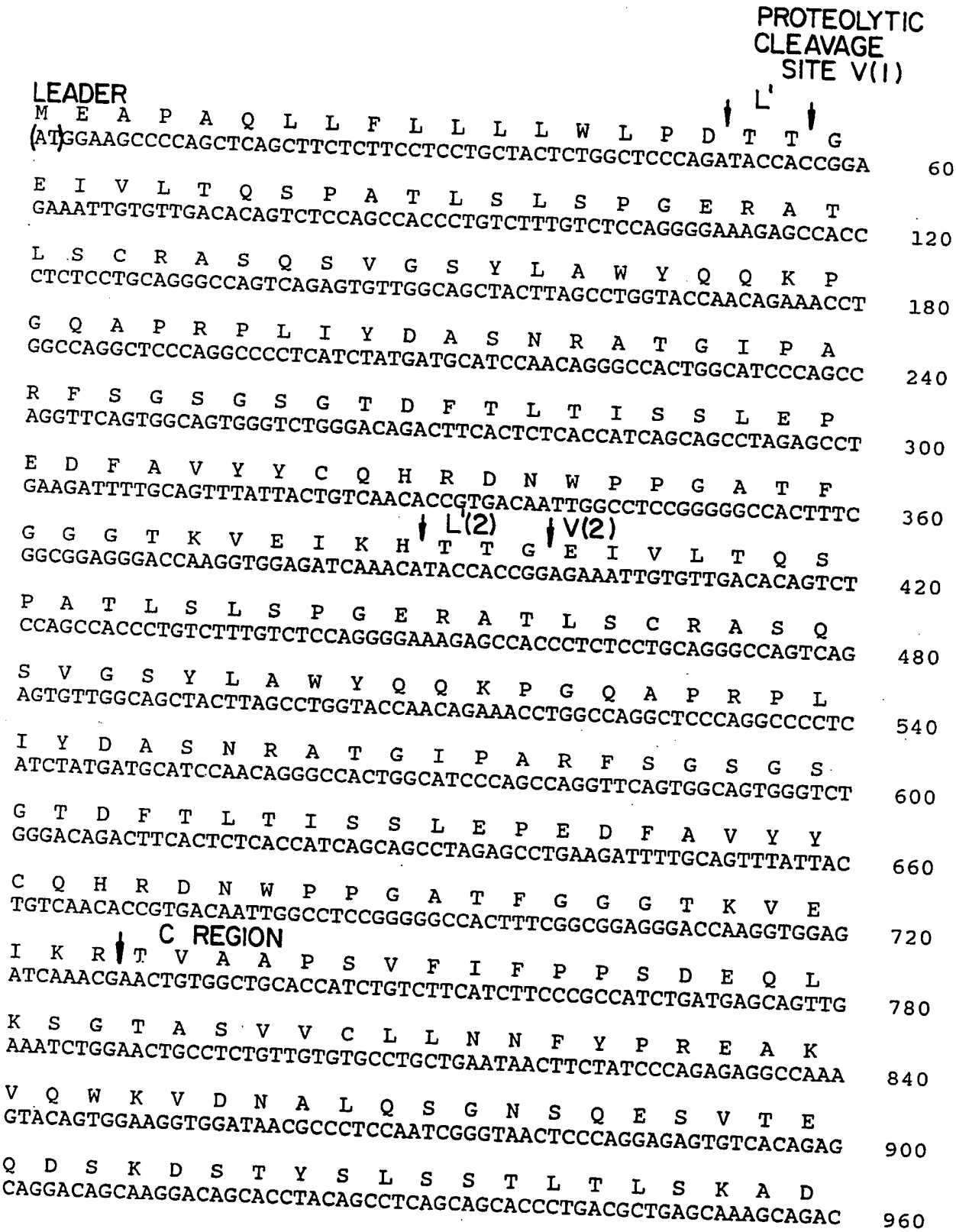


FIG._16.
(1 OF 2)

SUBSTITUTE SHEET

```
Y  E  K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC 1020

T  K  S  F  N  R  G  E  C  *  R  E  K  C  P  H  L  L  L  S
ACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCCACCTGCTCCTCAGT 1080

S  S  L  T  P  S  H  P  L  A  S  D  P  F  S  T  G  D  L  P
TCCAGCCTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTTCCACAGGGGACCTACCC 1140

L  L  R  S  S  S  S  S  F  T  S  P  P  S  S  S  L  A  L  I
CTATTGCGGTCCTCCAGCTCATCTTTCACCTCACCCCCCTCCTCCTCCTTGGCTTTAATT 1200

M  L  M  L  E  E  N  E  *  I  K  *  I  F
ATGCTAATGTTGGAGGAGAATGAATAAATAAAGTGAATCTTTGC                  1244
                      poly(A)
```

FIG._16.
(2 OF 2)

```
K  V  Y  L  E  D  *  V  L  T  D  S  S  F  P  S  I  Q  *  C
 R  C  I  W  K  I  K  F  *  Q  T  P  H  F  H  R  S  N  N  A
  G  V  S  G  R  L  S  S  N  R  L  L  I  S  I  D  P  I  M  H
AAGGTGTATCTGGAAGATTAAGTTCTAACAGACTCCTCATTTCCATCGATCCAATAATGC          60

T  *  G  D  D  W  A  Y  *  G  *  E  E  R  N  E  N  T  A  F
 L  R  E  M  T  G  H  I  E  D  R  K  R  E  M  K  T  Q  P  F
  L  G  R  *  L  G  I  L  R  I  G  R  E  K  *  K  H  S  L  L
ACTTAGGGAGATGACTGGGCATATTGAGGATAGGAAGAGAGAAATGAAAACACAGCCTTT          120

Y  I  V  L  N  R  L  V  P  N  I  I  W  V  N  L  G  D  *  G
 I  L  F  L  T  G  L  C  Q  T  S  S  G  *  I  *  V  I  E  E
  Y  C  S  *  Q  A  C  A  K  H  H  L  G  E  F  R  *  L  R  R
TATATTGTTCTTAACAGGCTTGTGCCAAACATCATCTGGGTGAATTTAGGTGATTGAGGA          180

E  E  R  H  R  N  E  I  L  *  A  Q  G  R  S  S  T  L  R  L
 K  K  D  I  G  M  K  F  S  E  H  K  G  E  V  L  H  S  D  *
  R  K  T  *  E  *  N  S  L  S  T  R  E  K  F  Y  T  Q  T  E
GAAGAAAGACATAGGAATGAAATTCTCTGAGCACAAGGGAGAAGTTCTACACTCAGACTG          240

S  Q  Q  T  F  L  A  *  Q  P  G  W  R  R  M  L  S  A  E  R
 A  N  R  L  F  W  P  D  N  Q  G  G  A  G  C  S  V  Q  R  G
  P  T  D  F  S  G  L  T  T  R  V  A  Q  D  A  Q  C  R  E  E
AGCCAACAGACTTTTCTGGCCTGACAACCAGGGTGGCGCAGGATGCTCAGTGCAGAGAGG          300

K  K  Q  V  V  S  A  A  G  S  S  A  P  T  Q  L  L  C  M  S
 R  S  R  W  S  L  Q  L  E  A  Q  L  P  P  S  C  F  A  C  P
  E  A  G  G  L  C  S  W  K  L  S  S  H  P  A  A  L  H  V  P
AAGAAGCAGGTGGTCTCTGCAGCTGGAAGCTCAGCTCCCACCCAGCTGCTTTGCATGTCC          360
                                                 OCTAMER
L  P  A  A  L  P  S  R  A  H  I  N  A  C  V  R  A  L  G  R
 S  Q  L  P  Y  L  P  E  P  I  S  M  P  V  S  E  P  W  G  G
  P  S  C  P  T  F  Q  S  P  Y  Q  C  L  C  Q  S  P  G  E  E
```

FIG._17.
(1 OF 3)

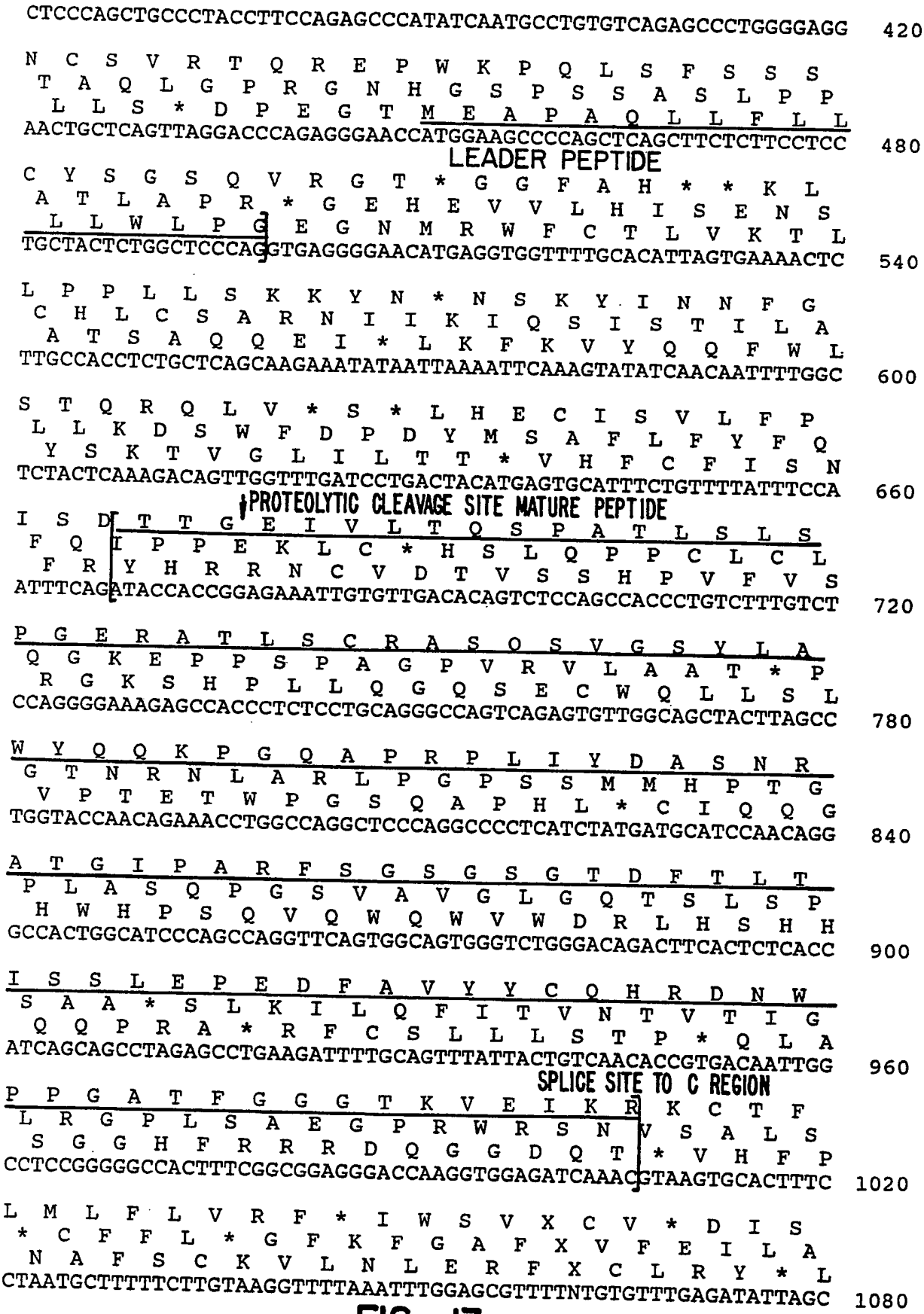


FIG._17.

(2 OR 3)

SUBSTITUTE SHEET

```
S  G  Q  F  Q  R  V  P  D  S  F  K  K  S  D  E  *  G  I  E
 Q  V  N  S  K  E  Y  Q  I  L  S  K  S  Q  M  S  K  G  *  K
  R  S  I  P  K  S  T  R  F  F  Q  K  V  R  *  V  R  D  R  K
TCAGGTCAATTCCAAAGAGTACCAGATTCTTTCAAAAAGTCAGATGAGTAAGGGATAGAA  1140

N  *  F  I  L  K  E  Q  P  S  A  R  Q  L  S  E  A  S  Q  C
 I  S  S  S  *  R  N  S  Q  A  L  G  S  *  V  R  H  L  N  A
  L  V  H  L  K  G  T  A  K  R  *  A  V  K  *  G  I  S  M  Q
AATTAGTTCATCTTAAAGGAACAGCCAAGCGCTAGGCAGTTAAGTGAGGCATCTCAATGC  1200

K
 R

AAGA                                                          1204
```

FIG._17.
(3 OF 3)

```
P  W  F  T  L  R  Y  L  G  K  *  Q  E  S  C  H  N  R  W  G
 R  G  S  L  C  G  I  *  V  N  N  K  K  V  V  I  T  D  G  E
  V  V  H  S  A  V  F  R  *  I  T  R  K  L  S  *  Q  M  G  N
CCGTGGTTCACTCTGCGGTATTTAGGTAAATAACAAGAAAGTTGTCATAACAGATGGGGA    60

I  L  Q  T  S  L  G  M  G  H  A  A  L  E  L  S  Q  G  R  C
 S  C  R  L  H  *  A  W  A  M  L  P  W  S  C  L  R  G  A  A
  P  A  D  F  T  R  H  G  P  C  C  P  G  V  V  S  G  A  L  P
ATCCTGCAGACTTCACTAGGCATGGGCCATGCTGCCCTGGAGTTGTCTCAGGGGCGCTGC   120

L  S  R  G  *  S  T  G  P  G  N  R  T  K  F  L  D  V  L  S
 S  P  E  V  R  A  Q  A  Q  V  I  G  L  N  F  *  M  C  Y  L
  L  Q  R  L  E  H  R  P  R  *  *  D  *  I  F  R  C  V  I  L
CTCTCCAGAGGTTAGAGCACAGGCCCAGGTAATAGGACTAAATTTTTAGATGTGTTATCT   180

*  T  H  C  T  T  A  V  F  S  M  *  I  I  S  C  K  I  *  H
 R  H  T  A  Q  L  L  C  S  L  C  K  L  S  P  V  K  Y  N  I
  D  T  L  H  N  C  C  V  L  Y  V  N  Y  L  L  *  N  I  T  L
TAGACACACTGCACAACTGCTGTGTTCTCTATGTAAATTATCTCCTGTAAAATATAACAT   240

*  S  L  H  *  I  Y  C  V  N  M  *  E  *  K  K  V  M  R  A
 E  A  C  I  K  Y  I  V  *  I  C  K  N  K  R  K  L  *  E  L
  K  P  A  L  N  I  L  C  K  Y  V  R  I  K  E  S  Y  E  S  *
TGAAGCCTGCATTAAATATATTGTGTAAATATGTAAGAATAAAAGAAAGTTATGAGAGCT   300

K  C  *  S  R  H  K  H  I  R  Y  N  Y  I  F  L  N  D  G  I
 S  V  N  Q  G  T  S  I  *  D  I  T  I  F  S  *  M  M  E  L
  V  L  I  K  A  Q  A  Y  K  I  *  L  Y  F  P  E  *  W  N  Y
AAGTGTTAATCAAGGCACAAGCATATAAGATATAACTATATTTTCCTGAATGATGGAATT   360

T  T  S  L  P  Q  D  T  S  S  A  L  S  P  A  S  P  Q  M  S
 L  P  V  S  P  R  T  L  H  L  P  *  A  Q  P  L  L  R  C  P
  Y  Q  S  P  P  G  H  F  I  C  P  E  P  S  L  S  S  D  V  P
ACTACCAGTCTCCCCCAGGACACTTCATCTGCCCTGAGCCCAGCCTCTCCTCAGATGTCC   420

H  P  E  L  A  I  *  W  G  T  C  K  *  G  P  P  S  T  D  E
 T  Q  S  L  L  Y  S  G  G  H  A  N  R  A  L  P  L  L  M  K
  P  R  A  C  Y  I  V  G  D  M  Q  I  G  P  S  L  Y  *  *  K
CACCCAGAGCTTGCTATATAGTGGGGGACATGCAAATAGGGCCCTCCCTCTACTGATGAA   480
                              OCTAMER
N  Q  P  S  P  D  P  A  A  L  G  E  E  P  S  T  R  S  R  R
 T  S  P  A  L  T  L  Q  L  W  E  R  S  P  A  L  E  V  G  G
  P  A  Q  P  *  P  C  S  S  G  R  G  A  Q  H  *  K  S  A  V
AACCAGCCCAGCCCTGACCCTGCAGCTCTGGGAGAGGAGCCCAGCACTAGAAGTCGGCGG   540
                                                    LEADER
C  F  H  S  V  I  S  T  E  H  R  G  L  T  M  E  F  G  L  S
 V  S  I  R  *  S  A  L  N  T  E  D  S  P  W  S  L  G  *  A
  F  P  F  G  D  Q  H  *  T  Q  R  T  H  H  G  V  W  A  E  L
TGTTTCCATTCGGTGATCAGCACTGAACACAGAGGACTCACCATGGAGTTTGGGCTGAGC   600

W  V  F  L  V  A  L  L  R  G  D  S  W  R  N  R  E  T  E  C
 G  F  S  S  L  L  F  *  E  V  I  H  G  E  I  E  R  L  S  V
  G  F  P  R  C  S  F  K  R  *  F  M  E  K  *  R  D  *  V  *
TGGGTTTTCCTCGTTGCTCTTTTAAGAGGTGATTCATGGAGAAATAGAGAGACTGAGTGT   660
```

FIG._18.
(1 OF 3)

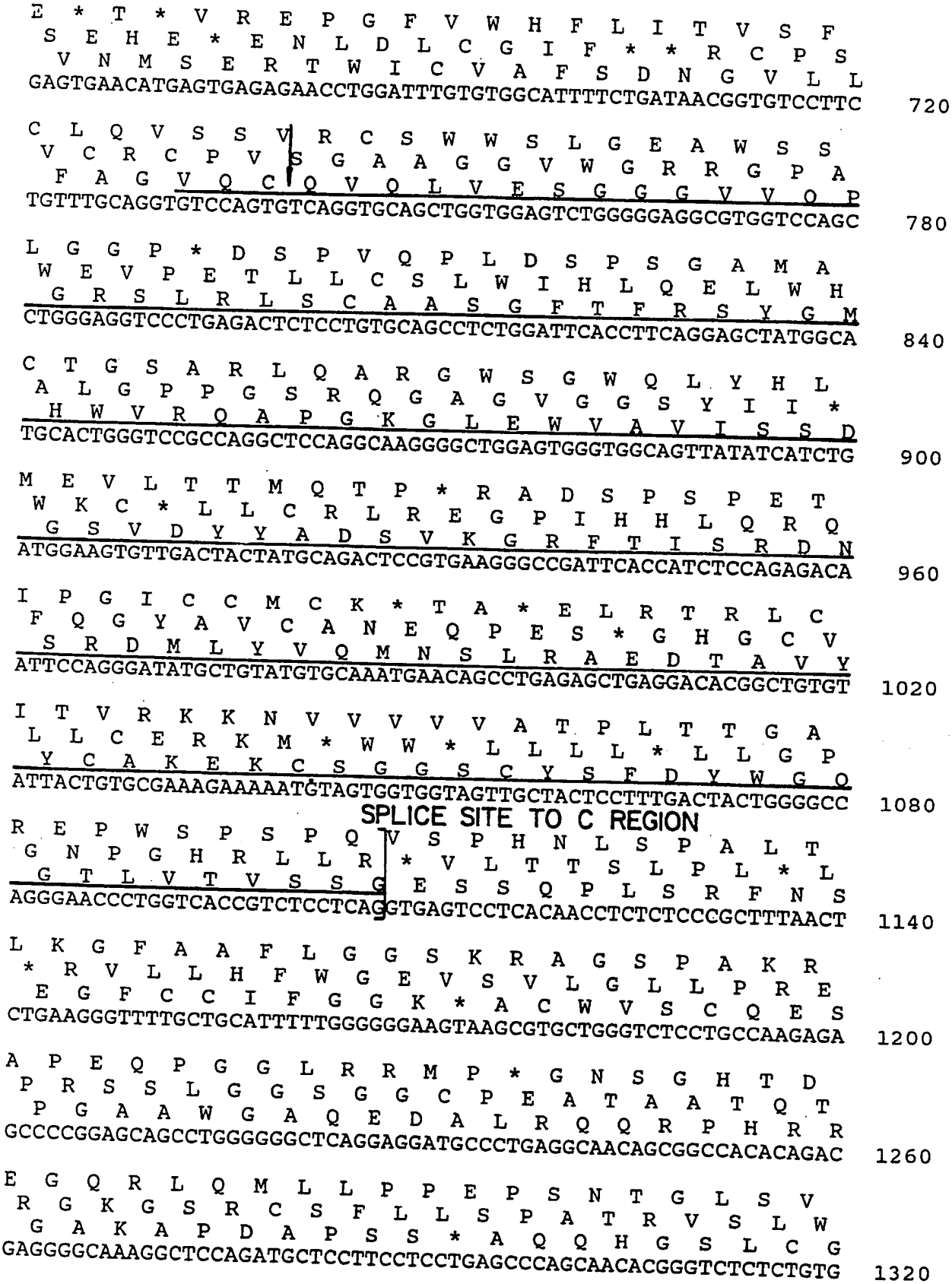


FIG._18.
(2 OF 3)
SUBSTITUTE SHEET

```
A  R  A  T  L  G  L  W  G  P  M  S  N  N  P  R  V  L  P  G
 P  G  P  P  W  A  S  G  V  Q  C  P  T  T  P  G  S  S  P  G
  Q  G  H  P  G  P  L  G  S  N  V  Q  Q  P  P  G  P  P  R  A
GCCAGGGCCACCCTGGGCCTCTGGGGTCCAATGTCCAACAACCCCCGGGTCCTCCCCGGG  1380

L  S  L  R  G  S  Q  G  L  S  G  V  P  V  L  A  W  G  P  G
 S  V  *  E  G  P  R  D  L  A  G  C  Q  F  L  P  G  V  L  A
  Q  S  E  R  V  P  G  T  *  R  G  A  S  S  C  L  G  S  W  H
CTCAGTCTGAGAGGGTCCCAGGGACTTAGCGGGGTGCCAGTTCTTGCCTGGGGTCCTGGC  1440

I  V  V  T  M  *  Q  L  V  R  P  L  G  P  G  N  P  G  H  R
 L  L  S  Q  C  D  N  W  F  D  P  W  G  Q  G  T  L  V  T  V
  C  C  H  N  V  T  T  G  S  T  P  G  A  R  E  P  W  S  P  S
ATTGTTGTCACAATGTGACAACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGT  1500

L  L  R  *  V  L  T
 S  S  G  E  S  S
  P  Q  V  S  P  H
CTCCTCAGGTGAGTCCTCACC                                          1521
```

FIG._18.
(3 OF 3)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06426

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 35/14; A61K 39/00;A61K 39/40; C12N 5/02; C12N 15/00
U.S. CL.:530/387; 424/85.8,87; 435/240.27; 935/12,15,99,102; 435/172.3

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	530/387, 424/85.8, 87, 435/240.27; 935/12, 15, 99, 102; 435/172.3

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

APS, CAS, Dialog

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	Journal of Immunology, volume 140, no. 8, issued 15 April 1988, S.H. Pincus et al, "Protective Efficacy of IgM Monoclonal Antibodies in Experimental Group B streptococcal infection is a function of antibody avidity" pages 2779-2785, see entire document.	1-6, 15-17,23 24,38-40
Y	US, A, 4,816,567 (Cabilly et al) 28 March 1989, see entire document.	6-14, 18-22, 33-37
X	US, A, 4,324,887 (Kasper) 13 April 1982, see entire document.	38-40

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
19 December 1990	22 FEB 1991
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Elizabeth Kleinman SPE for Suzanne Ziska ebw

Form PCT/ISA/210 (second sheet) (May 1986)

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